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FAO FOOD AND NUTRITION PAPER

41/9

Residues of some veterinary drugs in animals and foods

Abamectin
Chlortetracycline and tetracycline
Clenbuterol
Cypermethrin
α-Cypermethrin
Moxidectin
Neomycin
Oxytetracycline
Spiramycin
Thiamphenicol
Tilmicosin
Xylazine

WORLD HEALTH ORGANIZATION

Food and Agriculture Organization of the United Nations

Residues of some veterinary drugs in animals and foods

FAO
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Monographs prepared by the
Forty-seventh meeting of the
Joint FAO/WHO Expert Committee
on Food Additives
Rome, 4-13 June 1996

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JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Rome, 4-13 June 1996

List of participants

Members

Dr. L.-E. Appelgren, Professor of Pharmacology, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, The Swedish University of Agricultural Sciences, Uppsala, Sweden

Dr. D. Arnold, Deputy Director, Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany

Dr. J. Boisseau, Director, Laboratory of Veterinary Drugs, National Centre of Veterinary and Food Studies, Fougères, France (*Chairman*)

Dr. R. Ellis, Director, Chemistry Division, Food Safety and Inspection Service, Department of Agriculture, Washington, DC, USA (*Joint Rapporteur*)

Dr. P.G. Francis, Russet House, West Horsley, Surrey, England

Dr. R.D. Furrow, Beltsville, MD, USA (*Joint Rapporteur*)

Dr. Z. Hailemariam, Head, Food and Beverage Quality Control, Environmental Health Department, Ministry of Health, Addis Ababa, Ethiopia

Dr. R.C. Livingston, Director, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA

Dr. J.D. MacNeil, Head, Food Animal Chemical Residues, Health of Animals Laboratory, Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada

Dr. B.L. Marshall, Counsellor Veterinary Services, New Zealand Embassy, Washington DC, USA

Prof. J.G. McLean, Pro Vice-Chancellor, Division of Science, Engineering and Design, Swinburne University of Technology, Hawthorn, Victoria, Australia (*Vice-Chairman*)

Dr. A. Pintér, Deputy Director General, National Institute of Hygiene, Budapest, Hungary

Prof. J. Palermo-Neto, Department of Pathology, Faculty of Veterinary Medicine and Technology of Animal Husbandry, University of Sao Paolo, Sao Paolo, Brazil

Dr. J.L. Rojas Martinez, Chief, Toxicology Section, National Centre for Diagnosis and Research in Animal Health, Ministry of Agriculture, San José, Costa Rica

Dr. P. Sinhaseni, Associate Professor, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

Dr. S. Soback, Head, National Residue Laboratory, Kimron Veterinary Institute, Ministry of Agriculture, Beit Dagan, Israel

Dr. R. Wells, Director, Research and Development, Australian Government Analytical Laboratories, Pymble, New South Wales, Australia

Dr. K. Woodward, Director of Licensing, Veterinary Medicines Directorate, Ministry of Agriculture, Fisheries and Food, Addlestone, Surrey, England

Secretariat

Dr. C.E. Cerniglia, Director, Division of Microbiology, National Center for Toxicological Research, Jefferson, AR, USA (*WHO Temporary Adviser*)

Dr. P. Chamberlain, Veterinary Medical Officer, Division of Technology, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Rockvill, MD, USA (*WHO Temporary Adviser*)

Dr. R. Fuchs, Head, Department of Experimental Toxicology and Ecotoxicology, Institute for Medical Research and Occupational Health, Zagreb, Croatia (*WHO Temporary Adviser*)

Dr. R.J. Heitzman, Science Consultant, Newbury, Berkshire, England (*FAO Consultant*)

Dr. R. Herbert, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA (*WHO Temporary Adviser*)

Dr. J.L. Herrman, Scientist, Assessment of Risk and Methodologies, International Programme on Chemical Safety, WHO, Geneva, Switzerland (*WHO Joint Secretary*)

Dr. P. Jenkins, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland (*Editor*)

Dr. K. Mitsumori, Chief, Third Section, Division of Pathology, Biological Safety Research Center, National Institute of Hygienic Sciences, Tokyo, Japan (*WHO Temporary Adviser*)

Dr. J. Paakkanen, Nutrition Officer, Food Safety and Additives, Food Quality Liaison Group, Food Quality and Standards Service, Food and Nutrition Division, FAO, Rome, Italy (*FAO Joint Secretary*)

Mrs. M.E.J. Pronk, Advisory Centre of Toxicology, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands (*WHO Temporary Adviser*)

Dr. L. Ritter, Executive Director, Canadian Network of Toxicology Centres, University of Guelph, Guelph, Ontario, Canada (*WHO Temporary Adviser*)

Dr. G. Roberts, Director, Toxicology Evaluation Section, Commonwealth Department of Human Services & Health, Canberra, ACT, Australia (*WHO Temporary Adviser*)

Dr. G.J.A. Speijers, Head of the Section Public Health of the Advisory Centre of Toxicology, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands (*WHO Temporary Adviser*)

Dr. S. Sundlof, Director, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA (*WHO Temporary Adviser*)

ABBREVIATIONS USED IN THIS DOCUMENTS

ADI	-acceptable daily intake
AUC	-area under concentration-time curve
Av.	-average
b.i.d.	-twice a day
BP	-British Pharmacopoeia
Bq	-Becquerel (one disint/sec)
BST	-bovine somatotropin
bw, BW	-body weight
°C	-degrees Celcius
^{14}C	-radioactive Carbon-14
C_{max}	-maximum concentration
CAP	-chloramphenicol
µCi	-microcuries of radioactivity
cm^3	-cubic centimeter
conc	-concentration
CTC	-chlortetracycline
CV	-coefficient of variation
d	-day
DPM, dpm	-disintegration per minute
ECD	-electron capture detector
e.g.	-for example
EP	-European Pharmacopoeia
eq or EQ	-equivalents
F	-female
FDA	-Food and Drug Administration
g	-gram
µg	-microgram
GC	-gas chromatograpby
GI	-gastrointestinal
GLC	-gas-liquid chromatography
GLP	-Good Laboratory Practice
GVP	-Good Veterinary Practice
h	-hour
^3H	-tritium
HPLC	-bigb performance liquid chromatograpby
i.e.	-that is
i.m., IM	-intra muscular
i.m.i.	-intra muscular injection
i.p., IP	-intra peritoneal
i.v., IV	-intra venous
k_{el}	-rate constant
kg	-kilogram
L or l	-litre
LC	-liquid chromatography
LOD	-limit of detection
LOQ	-limit of quantitation
LSC	-liquid scintillation counting
M	-molar or mole
M	-male
max	-maximum
µm	-micrometer
mg	-milligram
min	-minute
ml	-millilitre
MR	-marker residue
MRL	-maximum residue limit
MRT	-mean residence time
MS	-mass spectrometry
n or No	-number
na	-not analyzed, assayed or available
nd, ND	-not detected
NER	-non extractable residues
ng	-nanogram
nm, NM	-not measured, if applicable
nm	-nanometer, if applicable
NMR	-nuclear magnetic resonance
NOEL	-no-observed-effect level
OTC	-oxytetracycline
ppb	-parts per billion
ppm	-parts per million
r	-regression coefficient
RIA	-radioimmunoassay
RSD	-relative standard deviation
SA	-Specific Activity
s.c.	-subcutaneous
SD	-standard deviation
SEM	-standard error of mean
sic	-correctly spelled
s.i.d.	-once a day
$t_{1/2}$	-half life
t_{max} or T_{max}	-time for maximum
TC	-tetracycline
TLC	-thin layer chromatography
TMS	-trimethyl silyl
TR	-total residues
TRA	-total radioactivity
TSD	-termionic specific detection
UD	-unchanged drug
USDA	-US Department of Agriculture
USP	-United States Pharmacopeia
UV	-ultraviolet
V_D	-volume of distribution
v/v	-volume/volume
wt	-weight
w/v	-weigbt/volume
WT	-withdrawal time
%	-per cent
>	-greater than
<	-less than
≤	-equal or less than

INTRODUCTION

The Monographs on the residues of the thirtheen eleven compounds contained in this volume were prepared by the forty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Rome, 4-13 June 1996. JECFA has evaluated veterinary drugs at previous meetings, including the 12th[1], 26th[2], 27th[3], 32nd[4], 34th[5], 36th[6], 38th[7], 40th[8], 42nd[9], 43rd[10], and 45th meetings.

In response to a growing concern about mass-medication of food producing animals and the implications for human health and international trade, a Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs was convened in Rome, in November 1984[12]. Among the main recommendations of this consultation were the establishment of a specialized Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) and the periodic convening of an appropriate body to provide independent scientific advice to this Committee and to the member countries of FAO and WHO. At its first session in Washington D.C. in November 1986, the newly-created CCRVDF reaffirmed the need for such a scientific body and made a number of recommendations and suggestions to be considered by JECFA[13]. In response to these recommendations , the thirty-second JECFA meeting was entirely devoted to the evaluation of residues of veterinary drugs in foods. Subsequently, the 34th, 36th, 38th, 40th, 42nd, 43rd, 45th, and 47th meetings of JECFA were also dedicated exclusively to evaluation of veterinary drugs.

The ninth session of the CCRVDF, held in Washington D.C. during December 1995, revised the priority list of veterinary drugs requiring evaluation. The drugs evaluated during the 47th meeting of JECFA included these compounds, except ceftiofur sodium, porcine somatotropin and spectinomycin.

The present volume contains summary monographs of the residue data on all of the thirteenth compounds on the agenda. The two β-adrenoceptor blocking agents, clenbuterol and xylazine, had not been evaluated before. The two anthelminthic agents, abamectin and moxidectin had been considered before by the 45th meeting of JECFA.

From the seven antimicrobial agents, chlortetracycline, oxytetracycline, tetracycline, neomycin and spiramycin had previously been evaluated by the Committee. The remaining antimicrobial agents, thiamphenicol and tilmicosin had not been evaluated before.

The insecticides, cypermethrin and α-cypermethrin, had not been considered before.

The pertinent information in each monograph was discussed and appraised by the entire Committee. The monographs are presented in a uniform format covering identity, residues in food and their evaluation, metabolism studies, tissue residue depletion studies, methods of residue analysis and a final appraisal of the study results. More recent publications and documents are referenced, including those on which the monograph is based. A summary of the JECFA evaluations from the 32nd to the present 47th meeting is included in Annex 1.

The assistance of the experts and FAO consultant in preparing these monographs is gratefully acknowledged.

REFERENCES

1. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some antibiotics (Twelfth Report of the Joint FAO/WHO Expert Committee on Food Additives), FAO Nutrition Meetings Report Series No. 45, 1969; WHO Technical Report Series No. 430, 1969.

2. Evaluation of Certain Food Additives and Contaminants (Twenty-Six Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 683; 1982.

3. Evaluation of Certain Food Additives and Contaminants (Twenty-Seventh Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 696; 1983.

4. Evaluation of Certain Veterinary Drug Residues in Foods. (Thirty-Second Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 763; 1988.

5. Evaluation of Certain Veterinary Drug Residues in Foods. (Thirty-Fourth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 788; 1989.

6. Evaluation of Certain Veterinary Drug Residues in Foods. (Thirty-Sixth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 799; 1990.

7. Evaluation of Certain Veterinary Drug Residues in Foods. (Thirty-Eighth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 815; 1991.

8. Evaluation of Certain Veterinary Drug Residues in Foods (Fortieth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 832; 1993.

9. Evaluation of Certain Veterinary Drug Residues in Foods (Forty-second Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 851; 1995.

10. Evaluation of Certain Veterinary Drug Residues in Foods (Forty-third Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 855; 1995.

11. Evaluation of Certain Veterinary Drug Residues in Foods (Forty-fifth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 864; 1996.

12. Residues of Veterinary Drugs in Foods, Report of a Joint FAO/WHO Consultation, Rome, 29 October - 5 November 1984. FAO Food and Nutrition Paper No. 32, 1985.

13. Report of the First Session of the Codex Committee on Residues of Veterinary Drugs in Foods. Washington D.C., 27-31 October 1986.

ABAMECTIN

First draft prepared by
Dr. J. Boisseau
National Agency for
Veterinary Medicinal Products
Fougères, France

ADDENDUM
to the Abamectin residue monograph prepared by the 45th meeting of the Committee and published in FAO Food and Nutrition Paper 41/8, Rome 1996

Abamectin is an agricultural compound approved as a plant protection agent which is used also as a veterinary drug for control of endo- and ectoparasites. The compound was evaluated as a pesticide by JMPR in 1992 and again in 1994 where an ADI of 0-0.2 μg per kg body weight was established. This ADI was based on a no-observed adverse effect level of 0.12 mg/kg of body weight using a safety factor of 500 because of concern about the teratogenicity of the Δ-8,9 isomer identified as an abamectin photodegradation product found in plant products.

Abamectin was on the agenda for the 45th JECFA in 1995 for evaluation of its use as a veterinary drug, intending to rely on the toxicological evaluation performed by the 1994 JMPR Meeting. On reviewing the data related to the use(s) of abamectin, the 45th JECFA concluded that the Δ-8,9 isomer is not present in animal tissues when abamectin is used as a veterinary drug. Therefore, the 45th meeting of JECFA recommended that consultations be held as soon as possible by JMPR and JECFA to resolve this problem. A joint JECFA/JMPR ad-hoc meeting was held in Geneva in September 1995 on this issue. The ad-hoc meeting recognized that consideration be given for a different ADI for abamectin as a pesticide and as a veterinary drug. As a consequence the JMPR meeting held in September 1995 agreed that the ADI of 0-0.2 μg/kg bw was not appropriate for abamectin residues in animal derived food that do not contain the Δ-8,9 isomer. In order to accommodate this situation, this JMPR meeting allocated an ADI of 0-1 μg/kg of body weight to abamectin on the basis of the NOEL of 0.12 mg/kg bw per day in the study of reproductive toxicity in rats and using a safety factor of 100 for veterinary drug use.

The JMPR meeting emphasized that MRLs that are recommended by JMPR and JECFA should be harmonized to include residues from the use of abamectin as a veterinary drug and the consumption by animals of fodder containing residues of abamectin.

The MRLs recommended by JMPR concerning cattle are the following

- muscle: 0.01 mg/kg
- liver, kidney: 0.05 mg/kg

Considering that:

- the ADI of 0-1 μg/kg bw established by JMPR results in a maximum allowable intake of residues of 0-60 μg for a 60 kg person
- Abamectin used as a veterinary drug is only intended for use in beef cattle
- Avermectin B_{1a} is considered as the appropriate marker residue
- Liver and fat are considered as the appropriate target tissues
- Abamectin does not lead to bound residues in fat tissues and that bound residues account for less than 15% in liver
- Avermectin B_{1a} accounts for 42% of the total residues in liver, 25% in fat tissue and 50% in kidney at 21 days post dosing

- There is an analytical method available

The committee recommended the following values for MRLs in cattle which, for abamectin used as veterinary drug, are expressed as avermectin B_{1a}:

- fat, liver: 100 µg/kg
- kidney: 50 µg/kg

As abamectin is only intended for beef cattle, there is no need for an MRL in bovine milk. Recognizing that liver, kidney and fat are the only tissues appropriate for monitoring residues of abamectin in animal tissues, there is no need for an MRL in bovine muscle where residues deplete to non-detectable concentrations at the recommended withdrawal time. Nevertheless, the JECFA recognized that JMPR has established MRL's for abamectin used as pesticide that are suitable for residues in cattle muscle and milk.

These MRLs result in a theoretical maximum daily intake of total residues of abamectin of 49 µg which, considering the total intake of 60 µg, gives an acceptable margin of safety for the possible additional ingestion of residues from pesticide use by consumption of fruits and vegetables and from the consumption of meat from cattle ingesting some contaminated fodder.

Tissue	MRL (µg/kg)	Factor TR/B_{1a}	TR (µg/kg)	Daily Food Intake (g)	Residues Consumed (µg B_{1a} eq)
Liver	100	100/42	238	100	24
Kidney	50	100/50	100	50	5
Fat	100	100/25	400	50	20
				Total	49

REFERENCE

Joint FAO/WHO Meeting on Pesticide Residues (JMPR): FAO Plant Production and Protection Paper 133, 1996

CHLORTETRACYCLINE AND TETRACYLINE

First draft prepared by
Dr. R.J. Wells
Australian Government Analytical Laboratories
Pymble, Australia

ADDENDUM
to the chlortetracycline and tetracycline monographs prepared by the 45th meeting of the Committee and published in FAO Food and Nutrition Paper 41/8, Rome 1996

Introduction

The 36th Joint FAO/WHO Expert Committee on Food Additives meeting in 1990 established MRLs for oxytetracycline of 600 μg/kg in kidney; 300 μg/kg in liver; 100 μg/kg in muscle; 100 μg/kg in milk; 200 μg/kg in eggs; and 10 μg/kg in fat[1] for all species for which residue depletion data were provided (cattle, swine, sheep, chickens, turkeys and fish). These MRLs were approved through the CODEX Alimentarius Commission in 1994.

An ADI of 0-3 μg/kg of body weight was assigned to oxytetracycline. The MRLs assigned by the Committee were based on the lowest values which could be monitored with the microbiological methods of analysis available at that time. Consequently, the 36th JECFA panel concluded that "the estimated maximum daily intake of oxytetracycline is 150 μg in milk, 30 μg in muscle, 0.5 μg in fat, 20 μg in eggs, 30 μg in liver, and 30 μg in kidney taking into account the food intake data as stated on page 9 of the 36th report of the Committee, WHO Technical Report Series 799, Geneva 1990, yielding a total of approximately 260 μg. This value slightly exceeds the ADI of 200 μg per person (one-tenth of the no-observed-effect-level of 2 mg per day). Since the ADI was derived from a conservative value of 2 mg per person per day and the consumption data are at the upper limit of the range for individual intake of animal products, the Committee concluded that the recommended MRLs do not present a risk for the consumer.

The MRL of 100 μg/kg recommended for milk contributed 150 μg to the theoretical food basket (daily consumption 1.5 l) and was the major factor in assuring that the ADI was exceeded by 30%.

The 45th Joint FAO/WHO Expert Committee on Food Additives meeting in 1995 allocated the same ADIs and MRLs, except milk, to chlortetracycline and tetracycline as those previously allocated to oxytetracycline at the 36th meeting, 100 μg/kg for muscle (cattle, pigs, poultry), 300 μg/kg for liver (cattle, pigs, sheep, poultry), 600 μg/kg for kidney (cattle, pigs, sheep, poultry), and 200 μg/kg for eggs (poultry). The MRLs were temporary pending further information as indicated below. Although the Committee realised that it is unlikely that tetracyclines will be used in combination, the MRLs allocated to the tetracyclines were defined as applying to both individual tetracyclines or the sum of the combined tetracycline residues. The ADI of 0-3 μg/kg of body weight previously assigned to oxytetracycline was converted to a group ADI with chlortetracycline and tetracycline at that meeting.

In arriving at its determination of MRLs, the 45th JECFA considered the recommendations of the 36th JECFA for oxytetracycline outlined above in combination with the decision to allocate a group ADI to CTC, OTC and TC. Target tissues for the analysis of all three tetracyclines were kidney and muscle in cattle, pigs and poultry and, based on limited data, kidney was the target tissue in sheep.

The following information was required for evaluation by the 47th JECFA in 1996:

1. The results of residue depletion studies in milk (cattle), in fat of cattle, pigs and poultry and in muscle, liver, kidney and fat of sheep in accordance with approved uses of these substances.

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[1] Note, the FAO Food and Nutrition Paper 41/3, incorrectly reports the MRL for fat as 100 μg/kg.

2. New and validated methods of analysis of chlortetracycline, oxytetracycline and tetracycline.

TISSUE RESIDUE DEPLETION STUDIES

General

It was inferred during discussions at the 45th JECFA that possibly specific formulations were both registered and used on a regular enough basis perhaps to warrant demands for extra residue data.

The report of the 45th JECFA reflected these discussions by requiring results of residue depletion studies in milk (cattle), in fat of cattle, pigs and poultry and in muscle, liver, kidney and fat of sheep in accordance with approved uses of these substances. The problems raised by this requirement is that the various formulations, particularly those used in milk production, were not specifically identified. Indeed, it was not certain if many of the formulations mentioned by several Committee members during discussion of chlortetracycline and tetracycline were registered or even currently available.

The information supplied is almost entirely derived by a re-culling of the initial extensive dossier supplied by Cyanamid. Furthermore, it deals exclusively with chlortetracycline data with no mention whatever of tetracycline residue data. Most of the information given below was included in the FAO Food and Nutrition Paper 41/8, but is reiterated here for the reader's easy reference.

Sheep

Two studies detail work on the depletion of chlortetracycline residues in liver, kidney and muscle tissues and in fat from sheep following dosing with 50 mg/kg, of chlortetracycline with and without 50 mg/kg of sulfamethazine (SMZ) in the feed for 42 Days.

Table 1. Depletion of Chlortetracycline Residues in Liver, Kidney, Muscle and Fat from Sheep Receiving 50 ppm of CTC with and without 50 ppm of Sulfamethazine (SMZ) in the Feed for 42 Days

Reference	Kohler and Abbey, 1971				Wang, 1971a			
CTC ppm in feed	50				50			
SMZ ppm in feed	0				50			
Withdrawal day	CTC mg/kg of Tissue				CTC mg/kg of Tissue			
	Liver	Kidney	Muscle	Fat	Liver	Kidney	Muscle	Fat
0	0.11	0.33	0.03	ND	0.21	0.39	0.04	ND
2	ND	ND-0.06	ND	ND	NM	NM	NM	NM
4	ND	ND	ND	ND	ND	0.04	ND	ND
6	NM	NM	NM	NM	ND	0.05	ND	ND
8	NM	NM	NM	NM	ND	ND	ND	ND

ND = Not Detected, below the sensitivity of the assay; NM = Not Measured

Milk

Soluble bolus formulations of chlortetracycline are used for vaginal or intrauterine administration in cows for reproductive infections. A study was conducted in which four lactating Holstein cows received intrauterine administration of four chlortetracycline soluble boluses (2 grams chlortetracycline) as a single treatment 1 to 3 days postpaltum. Average blood concentrations of chlortetracycline peaked at 0.149 mg/kg four hours after treatment, dropped below 0.05 mg/kg by day 3 post-treatment, and were not detected at 5 and 7 days post-treatment. Average levels of chlortetracycline in milk peaked at 0.146 mg/kg on day 1 post-treatment, dropped below 0.05 mg/kg by day 3 post-treatment, and were not detectable at 5, 6 and 7 days after treatment (Goodale, 1988a).

Residue data are available for two intramammary infusion products used for treatment of mastitia. The first study was conducted using an infusion product containing 426 mg of chlortetracycline per 6 mL syringe. One syringe was infused in each of the four quarters of the udder, and milk samples were assayed at 12-hour intervals until 120 hours post-medication. The 12-hour post-medication milk showed the highest activity, averaging 70 mg/kg chlortetracycline at that time. All milk samples were still positive at 96 hours post-treatment (average 0.07 mg/kg chlortetracycline). Four of the six cows still showed low activity (0.012 to 0.03 mg/kg) at the final sampling 120 hours post-treatment (Hewell, 1967). The second study was conducted with TARGOT® mastitis suspension containing 200 mg of chlortetracycline, 100 mg neomycin sulfate and 100 mg of dihydrostreptomycin sulfate (the latter two measured as base) per 6 mL syringe. One syringe was infused in each quarter of the udders of 10 clinically normal Dairy Friesians yielding approximately two gallons of milk daily. Individual cow milk samples were taken at 12-hour intervals for 144 hours after treatment. All milk samples contained less than 0.03 mg/kg chlortetracycline/mL at 120 hours after infusion and less than 0.125 mg/kg dihydrostreptomycin sulfate-neomycin sulfate (combined assay) at 72 hours after treatment (Nelson, 1968). A summary of the mean milk chlortetracycline levels are shown in Table 2.

Table 2. **Mean Milk Chlortetracycline Levels from 10 Cows Dosed with TARGOT® Mastitis Suspension Containing 200 mg of Chlortetracycline, 100 mg Neomycin Sulfate and 100 mg of Dihydrostreptomycin Sulfate**

Hours post infusion	Mean chlortetracycline level (mg/L)	Assay limit (mg/L)
0	<0.03	0.03
12	34.01	1.25
24	16.78	0.75
36	5.0	0.48
48	1.1	0.06
60	0.49	0.06
72	0.19	0.06
84	0.05	0.03
96	0.04	0.03
108	0.035	0.03
120	<0.03	0.03

Studies have shown that milk from cows receiving 0.22 mg chlortetracycline/kg b.w. daily by feed medication has no detectable chlortetracycline residues (Henderson, 1953; Shor et al, 1959). When the feeding level of

chlortetracycline was increased to 1.1 or 2.2 mg/kg b.w. daily, small amounts (up to 0.23 mg/L) were found in the milk. After 48 hours withdrawal of medicated rations, all milk samples were again negative. The sensitivity of the assay was 0.01 mg/L.

Cattle

Drain (1966a) report results of feeding 2.78 mg of CTC/kg to cattle for 30 days. Chlortetracycline residue levels in kidney and liver reached 0.37 and 0.16 mg/kg respectively whereas fat levels never exceeded the assay reporting level of 0.025 mg/kg. Similar negative fat results were obtained by Langner (1976) by feeding 1.01 mg of CTC/kg to cattle for 28 days and Colavita (1967) by feeding 2.01 mg of CTC/kg to cattle for 29 days.

The depletion of chlortetracycline from edible tissues of calves following a 10-day treatment at a dose of 22 mg/kg b.w. daily is presented in Table 3. These were young calves, averaging 42 kg b.w., receiving a milk replacer diet with medication supplied by soluble boluses once daily. Residues at zero-day withdrawal were highest in kidney, followed by liver, muscle and fat. After ten days withdrawal, residues of 0.06 to 0.15 mg/kg and 0.14 to 0.16 mg/kg remained in liver and kidney tissue, respectively. As has been shown in other species, the kidney and liver can be considered the target tissues.

Table 3. Depletion of Chlortetracycline Residues from Tissues of Calves Following Oral Treatment at 22 mg/kg bw Daily for 10 Days (DeLay, 1973)

Withdrawal day		Chlortetracycline, mg/kg of Tissue			
		Muscle	Liver	Kidney	Fat
0	Average	1.26	3.22	4.57	0.49
0	Range	1.08-1.55	2.70-3.65	4.30-4.90	0.31-0.63
3	Average	0.47	1.39	1.26	0.15
3	Range	0.38-0.59	1.11-1.80	1.00-1.55	0.10-0.20
7	Average	0.14	0.27	0.45	0.04
7	Range	0.07-0.21	0.12-0.46	0.24-0.70	0.03-0.06
10	Average	0.03	0.09	0.15	Neg-0.03
10	Range	0.02-0.04	0.06-0.10	0.14-0.16	Neg-0.04

Neg = Negative, below the sensitivity of the assay.

A summary of recent chlortetracycline depletion studies from liver and kidney of young calves following therapeutic doses of the drug from various dosage formulations for 7 consecutive days is shown in Table 4.

The calves in two of the studies received a diet of whole milk (Berger, 1989b; Goodale, 1988c), while in the other two studies the calves received a diet of reconstituted milk replacer (Rooney, 1988b, 1989b)). The daily doses of chlortetracycline ranged from 13.3 to 30.2 mg/kg bw. Residues of chlortetracycline at zero-day withdrawal were not directly proportional to the administered dose. The comparative results at day zero withdrawal between bolus formulation, where the average daily dose of 21.7 mg/kg exceeded the average of 13.3 mg/kg given in a soluble powder formulation are particularly intriguing. The soluble powder gave liver and kidney residue values of 13.7 and 19.2 mg/kg, well in excess of those from the bolus formulation (1.82 and 2.18 mg/kg in liver and kidney respectively). No ready explanation could be advanced for such disparate

results. Residues from liver and kidney samples did not exceed 0.05 mg/kg after the 25-day withdrawal or the 45-day withdrawal respectively. Although not shown in Table 4, no detectable chlortetracycline residues were found in fat samples after the zero-day withdrawal.

Table 4. Chlortetracycline Residue Depletion in Liver and Kidney Tissues of Calves Following Various Oral Dosing Forms for 7 Days

Reference	Berger, 1989b	Goodale, 1988c	Rooney, 1988b	Rooney, 1989b
Formulation	A-20	B	MA-200	SP
Calf weight, kg	38.4	46.1	43	41.3
Dose, mg/kg/d	30.2	21.7	16.3	13.3
Withdrawal day	Chlortetracycline, mg/kg of Liver Tissue			
0	16.7	1.82	6.5	13.7
15	NM	NM	0.073	NM
20	0.125	NM	0.075	NM
25	0.069	0.038	NM	ND-0.043
30	NM	ND-0.029	NM	NM
Withdrawal day	Chlortetracycline, mg/kg of Kidney Tissue			
0	25.3	2.18	9.7	19.2
15	NM	NM	1.09	NM
20	0.232	NM	0.092	NM
25	0.101	0.058	ND	0.059
30	NM	ND-0.039	NM	NM

Formulation: A-20 = AUROFAC 20 with neomycin and electrolytes in milk; B = CTC soluble boluses; MA200 = AUROFAC 200 MA in milk replacer; SP = CTC soluble powder in milk replacer; NM = Not Measured; ND = Not Detected

In summary, it should be noted that it is only at very high chlortetracycline dosing levels that fat residues are found and that these residue are 10-fold lower that the residues found in kidney and liver. No residues have been detected in fat at a withdrawal time where kidney tissue meets the assigned group MRL for tetracyclines.

Pigs

Data from studies in which pigs received 110 mg/kg chlortetracycline in feed for periods of 31 and 98 consecutive days are summarised in Table 5. When 330 mg/kg chlortetracycline was fed for a 98-day period (Berger, 1966b - see FNP 41/8, p. 51), residue levels of chlortetracycline were about twice those for pigs fed 110 mg/kg chlortetracycline for the same time (Sass and Messersmith, 1964; Stoner, 1962b). Drug levels in fat were more than 10 times lower than found in kidney and liver at early stages of withdrawal and chlortetracycline could not be detected after 5 days following withdrawal of treatment.

Table 5. **Chlortetracycline Residue Depletion in Liver, Kidney and Fat Tissues of Pigs Which Received 110 mg/kg chlortetracycline in Feed for 31 and 98 days**

Reference	Stoner, 1962h			Sass and Messersmith, 1964		
Days on Medication	31			98		
Weight of Pig, kg	33.6			83.8		
Drug in Feed, mg/kg	110			110		
Withdrawal day	Chlortetracycline, mg/kg					
	Liver	Kidney	Fat	Liver	Kidney	Fat
0	0.85	1.01	0.05	0.35	0.39	ND
3	0.09	0.15	0.01	NM	NM	ND
5	0.08	0.15	ND	ND-0.04	0.06	ND
7	0.08	0.14	ND	0.04	0.1	ND
10	NM	NM	NM	ND-0.04	0.04	ND

NM = Not Measured; ND = Not Detected, below the sensitivity of the assay.

Residue depletion data for edible tissues of pigs fed 440 mg/kg chlortetracycline in feed for 14 days is presented in Table 6. These data demonstrate that swine are similar to other species in that the highest and most persistent residues occur in kidney and liver tissue but are more than 10-fold lower in fat (Berger,1983).

Table 6. **Chlortetracycline Residue Depletion in Tissues from Pigs Which Received 440 mg/kg Chlortetracycline in Feed for 14 Days (Berger,1983)**

Withdrawal day	Chlortetracycline, mg/kg of Tissue			
	Muscle	Liver	Kidney	Fat
0	0.75	1.88	>3.78	0.2
1	0.28	0.65	1.69	0.06
3	0.23	0.68	1.5	0.06
4	0.14	0.53	0.8	0.04

Additional studies have been conducted in which 300 and 400 mg/kg chlortetracycline in feed were given to pigs for 7 consecutive days (Gingber, 1990d). As shown in Table 7, levels of chlortetracycline in fat were very much less than those in liver and kidney and were not detected 5 days after withdrawal.

Table 7. **Chlortetracycline Residues Depletion in Liver, Kidney and Fat Tissues of Pigs which Received 300 to 400 mg/kg Chlortetracycline in Feed for 7 Days**

Reference	Gingher, 1990d					
CTC, mg/kg Feed	300			400		
Withdrawal day	Chlortetracycline, mg/kg					
	Liver	Kidney	Fat	Liver	Kidney	Fat
0	1.23	2.29	0.08	1.32	2.69	0.10
3	0.109	0.121	ND	0.111	1.48	ND-0.02
5	0.102	0.087	ND	0.083	0.107	ND
7	0.069	0.08	ND	0.067	0.069	ND
10	0.058	0.06	ND	0.034	0.047	ND
12	ND-0.067	0.041	ND	ND-0.049	0.047	ND
15	0.036	0.038	ND	0.046	0.048	ND
20	ND-0.034	ND-0.035	ND	ND-0.037	0.035	ND
25	NM	NM	ND	NM	NM	ND
30	NM	NM	ND	NM	NM	ND

NM = Not Measured; ND = Not Detected, less than 0.025 mg/kg of tissue

Poultry

Chickens

Two separate studies (Drain, 1962a ; Gingher, 1980) in older chickens adminstered 220 mg/kg chlortetracycline in the feed showed residue levels of 0.66 and 0.71 mg/kg in liver and 0.42 and 0.75 mg/kg in kidney, respectively, at day 0 after withdrawal of medication compared to levels of 0.02 and 0.04 mg/kg in skin with adhering fat. At day 1 withdrawal no residues of chlortetracycline were detected in fat. Similarly, Gingher (1979) found no chlortetracycline residues in fat of chickens, fed medicated diets of 110 ppm chlortetracycline with added monensin for 51 days, 1 day after withdrawal of medication.

A more recent study conducted with 300 mg/kg chlortetracycline in feed for a 7-day treatment period (Gingher, 1988b) to chickens followed a similar trend. Liver tissues contained 0.328 mg/kg of chlortetracycline at the zero-day withdrawal point, while kidney tissues contained 2.45 mg/kg chlortetracycline. Residues in skin with adhering fat were 0.078 mg/kg at 0 day withdrawal and were below 0.025 mg/kg at one day withdrawal.

A summary of residue data from chickens treated via the drinking water at level of 120 mg/kg for a period of 7 days is shown in Table 8 (Gingher, 1989a). Liver is essentially free of chlortetracycline residues two days after withdrawal, while no measurable amounts of chlortetracycline persist in fat one day after withdrawal.

Table 8. Residues in Liver and Fat Tissues From Chickens Receiving Chlortetracycline in the Drinking Water

Reference	Gingher, 1989a	
CTC in Water, mg/kg	120	
Days on Medication	7	
	Chlortetracycline, mg/kg of Tissue	
Withdrawal day	Liver	Fat
0	0.276	0.1
1	ND-0.049	ND
2	ND-0.03	ND
3	ND	ND
4	ND	ND

ND = Not Detected, less than sensitivity of method

Turkeys

Turkey poults were fed medicated feed at a concentration of 0 and 400 g/ton chlortetracycline in a low calcium diet from one day old to 21 days of age. Tissues and blood was collected from 0 to 5 days after withdrawal of medication. The limit of detection of the microbiological assay was 0.05 mg/kg for liver and 0.025 for muscle, fat and kidney (Drain, 1961).

Average residue levels in fat were:

Withdrawal day	Average residue level (mg/kg)
0	0.47
1	0.17
2	0.09
3	0.085
4	0.075
5	0.057

Fifteen week-old turkeys were medicated with chlortetracycline as a soluble powder in the drinking water to provide 55 mg CTC/kg for 14 days. Tissues were measured at 0, 6, 12, 24 and 36 hours after withdrawal using a microbiological assay. Average fat levels at zero hour withdrawal were 0.047 mg/kg in males and 0.025 mg/kg in females. Levels fell below the limit of quantification after 6 hours.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Microbiological Methods

There has been no general improvement in sensitivity from any reported validated microbiological method since the review of oxytetracycline at the 36th JECFA where quantitation levels of 100 μg/kg were established. In establishing these levels of quantification, the 36th JECFA allowed a safety margin of twice the levels attainable, thus levels of 50 μg/kg were achieved in the validated antimicrobial assay submitted to the Committee (J. Boisseau, comment made at the 45th JECFA). Table 28 of the monograph on Chlortetracycline in the *FAO Food and Nutrition Paper 41/8* is reproduced here as Table 9 to show the comparative results from the analysis of porcine kidneys for chlortetracycline using both chemical (HPLC) and antimicrobial assay methods (MB).

It should be noted that the results for both methods are comparable and that the limit of quantification is 20 μg/kg. However, such limits of quantification by microbiological assay are not achievable for either oxytetracycline or tetracycline.

The different levels to which the tetracyclines are able to be detected and quantified in a microbiological assay raises another problem when bioassay is used as the sole regulatory method of residue analysis. In a routine laboratory assay in muscle, the Australian Government Analytical Laboratories attains quantification limits (LOQ) of 100 μg/kg for oxytetracycline and tetracycline in both muscle and milk whereas chlortetracycline is at least 3 times more sensitive in the same tests. Limits of Detection (LOD) are 3 times lower than LOQs and would allow detection of residues of all three antibiotics in the target tissues of kidney and muscle as well as in milk at levels of 50 μg/kg.

Microbiological assays, despite being a cost effective method to monitor antibiotic residues, are not able to yield positive identification of the residue(s) detected. The allocation of a group ADI to three tetracyclines required that the MRLs assigned were defined as applying to both individual tetracyclines or the sum of the combined tetracycline residues. Under these circumstances, residue methods are required which identify individual tetracyclines but no microbiological assay will meet this criterion.

Therefore it becomes mandatory to employ a chemical method for regulation of tetracyclines usage. However, a general microbial inhibition procedure will prove a useful and cost effective preliminary screen prior to identification and quantification by chemical analysis.

Chemical Methods

Modern chemical methods of tetracycline antibiotic analysis individually identify and quantify all three tetracyclines discussed here at levels at or below the MRLs allocated to the tetracyclines. Indeed, some methods published in the last few years reach levels of detection and quantification in milk which would readily allow a lowering of the MRL of OTC in milk to 50 μg/kg and establishment of MRLs for chlortetracycline and tetracycline at the same level. Methodology has been reviewed in the monograph on Chlortetracycline in the *FAO Food and Nutrition Paper 41/8.*

In general, residues of oxytetracycline and tetracycline are more readily recovered and quantified by HPLC than are residues of chlortetracycline. Recoveries of oxytetracycline and tetracycline in both muscle and milk are typically 10-20% higher than for chlortetracycline. However, CTC can be detected at concentrations 3 times lower than can either OTC and TC in the microbiological inhibition methods.

A collaborative study of the Farrington-Carson method for tetracycline analysis has been reported for milk (Carson et al, 1996). Eight laboratories analysed known control and fortified milk sample for 7 tetracyclines including chlortetracycline , oxytetracycline and tetracycline. At fortification levels of 15 μg/l, mean recoveries (%RSD) were 61.7% (25.4) for CTC, 75.2% (12.5) for OTC and 73.6% (12.6) for TC. At fortification levels of 30 μg/l, mean recoveries (%RSD) were 64.2% (7.4) for CTC, 77.5% (8.2) for OTC and 74.8%(8.7) for TC. Results from these studies meet the Center for Veterinary Medicine (CVM, US FDA) guidelines for

accuracy and precision for residue analysis at this concentration target level. The method is free from analytical interferences and is able to accommodate large sample numbers in routine use.

Table 9. **Comparison of Microbiological Assay and HPLC Analysis for Chlortetracycline Residues in Kidneys from Pigs which Received 300 to 400 mg/kg in Feed for 7 Days (Gingher, 1990d)**

CTC, mg/kg Feed		300	300	400	400
Assay Method		MB	HPLC	MB	HPLC
Withdrawal Day		Chlortetracycline, mg/kg of Kidney			
0	Average Range	2.29 1.45-3.35	1.925 1.029-3.023	2.69 1.64-3.15	2.255 1.362-2.773
3	Average Range	0.121 0.108-0.129	0.101 0.095-0.114	0.148 0.074-0.245	0.124 0.062-0.221
5	Average Range	0.087 0.072-0.124	0.068 0.051-0.097	0.107 0.077-0.153	0.082 0.055-0.111
7	Average Range	0.08 0.067-0.100	0.054 0.042-0.074	0.069 0.058-0.087	0.049 0.039-0.060
10	Average Range	0.06 0.050-0.070	0.04 0.034-0.044	0.047 0.039-0.053	0.029 0.023-0.034
12	Average Range	0.041 0.029-0.070	0.024 <0.02-0.033	0.047 0.030-0.062	0.031 0.020-0.043
15	Average Range	0.038 0.032-0.050	0.023 <0.02-0.03	0.048 0.037-0.060	0.03 0.022-0.039
20	Average Range	ND-0.035 ND-0.046	<0.02 <0.02-0.029	0.035 0.031-0.040	0.023 <0.02-0.034

More recent work includes a collaborative study (MacNeil et al, 1996) between 13 laboratories using the Oka method, first published in 1985. This study was conducted both on fortified (50 μg/kg) and incurred samples of porcine and bovine muscle and kidney. In general oxytetracycline and tetracycline could be more readily quantified at lower levels than could chlortetracycline. It was concluded that tetracycline residues could be successfully analysed in the animal tissues tested at levels of 100-600 μg/kg depending on the analyte-tissue combination. Although the equipment required is available in most standard analytical laboratories, the methodology is not exceptionally robust and is dependent on the availability of disposable cartridges from analytical suppliers which meet necessary performance criteria which are not always achieved. It also appears that more experienced laboratories attain detection limits and analyte recoveries well ahead of less adept laboratories. Some problems in this study arose from the manipulations required in the fortification of samples and results reported must represent a poorer outcome than would be attained by any one laboratory working on incurred samples.

JECFA Requirement for New and Validated Methods of Tetracycline Analysis

The 45th JECFA required new and validated methods of tetracycline analysis to be submitted for evaluation by the 47th JECFA in 1996.

It appears that, in setting this requirement, the 45th JECFA Committee was focussing on the need to readily monitor milk and milk products to lower levels than presently possible by microbiological methods. Certainly published methods allow quantification of tetracyclines in other tissues at levels consistent with the assigned MRLs. A more sensitive new microbiological assay or the introduction of an immunochemical method for milk might therefore be a sufficient requirement of the sponsor.

However, a review of published methods for tetracycline analysis suggests that allocated MRLs for tetracylines can be satisfactorily monitored by a combination of the microbiological (screening for antibiotic residues) and chemical (identification and quantification) analyses presently available.

Although it is not the function of JECFA to advise on the methodology to be pursued, the introduction of an immunochemical method might be appropriate in this case. Immunochemical methods such as ELISA could well be an easily applicable alternative to or could be used in conjunction with a microbiological method and might overcome the difference in microbiological response for different tetracyclines in antimicrobial assays. Such methods are presently commercially available and tend to be substance rather than class specific. One such immunochemical method which has had its performance claims validated by the Association of Official Analytical Chemists (AOAC International) is discussed below.

The results of this validation study meet the JECFA requirements for a sufficiently sensitive new validated method to be presented to the 47th JECFA in 1996 and are therefore presented in some detail.

Validation of a Commercial Test Kit for Tetracyclines in Milk

The AOAC Research Institute has performed validation studies of a commercially available test kit for chlortetracycline, oxytetracycline and tetracycline (Charm Sciences Inc. Test Kit for Tetracyclines in Milk: AOAC Research Institute Report, 1996). The assay is based on a competitive radioimmuno-assay between the target tetracycline and ^{3}H-tetracycline using antibodies bound to microbial receptors which are specific only for tetracyclines.

The test kits results were compared with those of chemical analysis conducted by an experienced independent laboratory for both selectivity and sensitivity. The kit gave no false positives for any of 60 negative control samples, easily meeting the criterion that a test kit should be at least 90% selective with 95% confidence. The kit also met the AOAC criterion of at least 90% sensitivity with a 95% confidence level at the claimed detection level for each of the tetracyclines tested as shown in Table 10.

Table 10. Charm II Tetracycline Drug Test Kit: 90% Sensitivity Level

Antimicrobial agent	90% Sensitivity level (μg/l)	US FDA Safe level (μg/l)
Chlortetracycline	28	30
Oxytetracycline	19	30
Tetracycline	5	80

Table 11 shows the results of a large series of analyses of milk for tetracyclines conducted with the test kit and performed by the independent testing laboratory. The results show that the ability of the test kit to detect residues of all three tetracyclines in milk exceeds that of the chemical analytical technique used by the independent testing laboratory to confirm these positive findings. It is noteworthy that in these studies both the test kit and the chemical method used to confirm test kit results have the ability to detect residues of chlortetracycline, oxytetracycline, and tetracycline at levels at or below 30 μg/L.

Table 11. **Comparison of Results from a Series of Analyses of Milk for Tetracycline Residues Conducted by Both the Charm II Tetracycline Drug Test Kit and an Independent Laboratory**

Substance	Concentration (μg/l)	Commercial Kit[a] Positive (out of 30)	Commercial Kit[a] Positive (%)	Independent Laboratory[b] Positive (out of 30)	Independent Laboratory[b] Positive (%)
CTC	5	2	7		
	6	3	10		
	9			9	30
	12	15	50	12	40
	16			15	50
	18	28	93		
	23			29	97
	24	30	100		
	30	30	100	29	97
OTC	3	1	3		
	6	4	13	2	7
	7			0	0
	10			16	53
	12	24	80		
	15			28	93
	18	28	93		
	24	30	100		
	30	30	100	30	100
TC	2			1	3
	3			15	50
	4	28	93	26	87
	6	30	100	30	100
	8	30	100		
	16	30	100		
	80	30	100	30	100

[a] Data resubmitted by kit manufacturer and reviewed by FDA's Center for Veterinary Medicine and the AOAC Research Institute; [b] Data collected by the University of New Hampshire

The accepted practice of herd administration of both chlortetracycline and oxytetracycline led to an additional sensitivity criterion in the US-FDAs 'Protocol for Evaluation of Milk Residue Screening for Drugs other than β-Lactams and Sulfonamides'. This specification requires that approved test kits produce no more than 10% positive results at a level that could be incurred in a farm bulk from a herd in which medicated feeds had been widely used. These levels were experimentally determined at 3 and 5 μg/L for oxytetracycline and chlortetracycline residues respectively. The Charm II test kit met these specifications with 3% and 7% positives for oxytetracycline and chlortetracycline respectively at the levels determined above.

The AOAC concluded that, used in accordance with agreed testing procedures, the Charm II Tetracycline Test Kit would 'be expected to produce significantly less than 1% false violative results for milk with low levels of oxytetracycline and chlortetracycline residues (sic).

The Committee recognised that the validated analytical methodology available for tetracyclines is of sufficient sensitivity to accommodate JECFA-allocated MRLs in tissues for all three tetracyclines. Moreover, a new interlaboratory study in milk and the availability of a commercial tetracycline test kit which has undergone rigorous comparison with a validated chemical method allows the reliable monitoring of tetracyclines in milk at levels well below 50 μg/kg.

APPRAISAL

A detailed comparison of chlortetracycline levels in fat and kidney of cattle, pigs, sheep and poultry at various times after withdrawal of medication indicated that residues of chlortetracycline in fat were at least 9 times lower than levels in kidney and depleted far more rapidly. Cattle fed 22 mg per kg of body weight chlortetracycline for 10 days had mean fat levels of 40 μg/kg 7 days after dosing, whereas mean values in kidney, liver and muscle were 450, 270 and 140 μg/kg, respectively. Pigs fed 400 mg per kg of body weight chlortetracycline for 7 days contained mean fat levels of 100 μg/kg at zero day withdrawal and mean residue values in kidney and liver of 2690 and 1320 μg/kg respectively. Two separate studies in chickens administered 220 mg/kg chlortetracycline in the feed showed residue levels of 660 and 710 μg/kg in liver and 420 and 750 μg/kg in kidney, respectively, at day 0 after withdrawal of medication compared to levels of 20 and 40 μg/kg in skin with adhering fat. At day 1 withdrawal no residues of chlortetracycline were detected in fat. Another study found no chlortetracycline residues in fat of chickens, fed medicated diets of 110 ppm chlortetracycline with added monensin for 51 days, 1 day after withdrawal of medication.

Due to the rapid depletion of tetracyclines in fat, the Committee concluded that fat is not an appropriate target tissue for this class of drug and recommend that the assignment of an MRL for fat is not required.

Recent HPLC chemical methods of tetracycline antibiotic analysis individually identify and quantify all three tetracyclines at levels at or well below the MRLs allocated to the tetracyclines. Two validation trials have been published in 1996 which clearly attain levels of detection and quantification in all tissues which allow regulation of assigned MRLs. Furthermore, a published validation study in milk demonstrates that current methodology would readily permit a lowering of the MRL of tetracyclines in milk to 50 μg/kg. This validated quantitative method is also supported by both microbiological and immunochemical screening methods with the requisite detection levels and performance characteristics.

Notwithstanding the capability of analytical methods to identify and quantify residues at a lower MRL in milk, the Committee retained an MRL of 100 μg/l for oxytetracycline and recommended this same milk MRL for chlortetracycline and tetracycline. In maintaining this milk MRL, the Committee considered data showing that for oxytetracycline, milk levels fell below 100 μg/l only after 6-8 milkings following intramammary infusion or 10-14 milkings following administration of long acting formulations. Data for chlortetracycline also show that at least 6-10 milkings would be necessary following administration of intramammary infusion formulations to ensure that no violative milk levels were encountered if an MRL of 100 μg/l were adopted. A lowering of the milk MRL to 50 μg/l would result in unacceptable withdrawal times for milk.

The Committee also reaffirmed the opinion of the thirty-sixth Committee that no risk to human health would result from the ADI of 180 μg/day being exceeded by 30%, if these MRLs previously established for oxytetracycline were also recommended for chlortetracycline and tetracycline.

Maximum Residue Limits

The Committee recommended that the MRLs for oxytetracycline of 600 μg/kg in kidney, 300 μg/kg in liver and 100 μg/kg in muscle of cattle, pigs, sheep, and poultry and of 100 μg/l in milk of cattle and sheep, and 200 μg/kg in eggs of poultry, be extended to chlortetracycline and tetracycline.

The Committee recommended that the MRL of 10 μg/kg for oxytetracycline in fat be withdrawn and that MRLs in fat for chlortetracycline and tetracycline are not required.

Based on the food basket used by the Committee, the theoretical maximum daily intake of chlortetracycline, oxytetracycline and tetracycline, used alone or in combination, would be 260 μg/day.

REFERENCES

Agososter, R.M.L. and Rasmussen, K.E. (1992). *J. Pharm Biomed Anal*, **10**, 349-354.

Alford, B.T. (1967). American Cyanamid Company Report **FD 15:** 1143-1235. Experiment P-66-21-FT.

Alford, B.T. (1970). American Cyanamid Company Report **FD 18:** 1143-1163, Experiment BB-66-7-FT.

AOAC Research Institute Report, (1996). Charm Sciences Inc. Test Kit for Tetracyclines in Milk, *J Assoc Off. Anal. Chem. International*, 19, 36A-39A.

Berger, H. (1965). American Cyanamid Company Report **FD 13:** 1-29. Experiment BB-64-4-FT.

Berger, H. (1966b). American Cyanamid Company Report **FD 14:** 634-680. Experiment P-64-14-FT.

Berger, H. (1967). American Cyanamid Company Report **FD 15:** (b) 466-492. Experiment P-66-14-FT, (c) 671-715. Experiment P-66-17-FT, (d) 319-369. Experiment BB-67-1-FT, (e) 1382-1447. Experiment A65-36-FT.

Berger, H. (1970). American Cyanamid Company Report **FD 18:** 2015-2126k. Experiment BB-66-10-FT.

Berger, H. (1971). American Cyanamid Company Report **FD 19:** 29-44. Experiment A-70-24-FT-1.

Berger, H. (1972). American Cyanamid Company Report **FD 20:** 87-140. Experiment A-67-21-FT.

Berger, H. (1974). American Cyanamid Company Report **FD 22:** 293-341. Experiment B-73-25FT.

Berger, H. (1982). American Cyanamid Company Report **FD 29:** (a) 1745-1781. Experiment A-8 1-1, (b) 1782-1814. Experiment A-81-2, (c) 1709-1744. Experiment L-1852.

Berger, H. (1983). American Cyanamid Company Report **FD 31:** 705-731 . Experiment P-82-16.

Berger, H. (1984). American Cyanamid Company Report **FD 32:** 1157-1255 . Experiment P-82-12.

Berger, H. (1989). American Cyanamid Company Report **FD 37:** (a) Report No. 7. Experiment P-88-7, (b) Report No. 5. Experiment B-88-16, (c) Report No. 6. Experiment P-88-7A, (d) Report No. 8. Experiment P-88-8A, (e) Report No. 9. Experiment P-88-8.

Berger, H. (1990). American Cyanamid Company Report **FD 38:** Report No. 68. Experiment P-89-27.

Berger, H. and T.R. Garces. (1981). American Cyanamid Company Report **FD 28:** 1347-1386. Experiment B-80-8.

Berte, F. and Vandoni, G. (1962). *Chemotherapia*, **5**, 219-230.

Biroc, S.E. (1992b). American Cyanamid Company Report **FD 40:** Report No. 16.00. Experiment L-2437.

Bradley, B.D., Allen, E.H., Showalter, D.H. and Colaianne, J.J. (1982). *J Vet Pharmacol Therap*, **5**, 267-278.

Broquist, H.P., and Kohler, A.R. (1954). *Antibiotics Annual* 1953-1954, 409-415.

Buyske, D.A., Eisner H.J. and Kelly, R.G. (1960). *J Pharm Exper Therapy*, **130**, 150-156.

Carson, M.C., and Breslyn, W., (1996). Simultaneous determination of multiple tetracycline residues in milk by metal chelate affinity chromatography. *J Assoc Off Anal Chem International*, **19**, 29-42.

Craig, S.L. (1992). American Cyanamid Company Report **FD 40:** Report No. 12.00. Experiment L-2453.

Colavita, J. (1967). American Cyanamid Company Report **FD 15:** 246-303. Experiment BB-66-1-FT.

DeLay, R.L. (1973). American Cyanamid Company Report **FD 21:** 732-77O. Experiment B-72-44-FT.

Drain, J.J. (1961). American Cyanamid Company Report **C.F. 6:** 17-22D, Experiment A-61-9-LT.

Drain, J.J. (1962). American Cyanamid Company Report **FD 10:** (a) 400-508. Experiment A-62-5-FT, (b) 510-699, Experiment A-62-5-FT, Phase II.

Drain, J.J. (1966). American Cyanamid Company Report **FD 14:** (a) 272-309. Experiment BB-65-8-FT, (b) 310-349. Experiment BB-65- 12

Eisner, H.J. and Wulf, R.J. (1963). *J Pharmacol Exp Ther*, **142**, 122-131.

Eisner, H.J., Stern, K.E., Dornbush, A.C. and Oleson, J.J. (1953). *J Pharmacol Exp Ther*, 442-449

Elliott, R.F. and Whitehill, A.R. (1957). *Proc Soc Exp Biol Med*, **94**,119-122

Farrington, W.H.H., Tarbin, J.A., Bygrave, J.and Shearer, G. (1991). *Food Additives and Contaminants*, **8**, 55-64.

Ferguson, E.F.. (1972). American Cyanamid Company Report, **B 539:** Experiment A-724-54-FT.

Flinter, G.F. (1963). *Journal of the American Medical Association*, **184**, 111.

Fluop, M and Drapkin, A. (1965). *New England J Med*, **9**, 986.

Garces, T.R. (1979). American Cyanamid Company Report **FD 29:** 1-58, Experiment P-80-11.

Gingher, P.E. (1979). American Cyanamid Company Report **FD 27:** 600-670, Experiment A-78-9b

Gingher, P.E. (1980). American Cyanamid Company Report **FD 28:** 131-197, Experiment B-809-15

Gingher, P.E. (1981). American Cyanamid Company Report **FD 29:** 150-186, Experiment A-79-4b

Gingher, P.E. (1988). American Cyanamid Company Report **FD 36:** (a) Report No. 106. Experiment A-87-39, (b)American Cyanamid Company Report **FD 36:** (a) Report No. 123. Experiment A-88-7.

Gingher, P.E. (1989). American Cyanamid Company Report FD 37: (a) Report No. 3. Experiment A-88-8, (b) Report No. I0. Experiment A-88-8A, (c) Report No. 12. Experiment A-88-11, Report No. 13. Experiment A-88-12, (e) Report No. 4. Experiment A-88-7A,

Gingher, P.E. (l990). American Cyanamid Company Report FD 38: (a) Report No. 28. Experiment P-89-26, (b) Report No. 56. Experiment A-89-10, (c) Report No. 73. Experiment P-89-28A, (d) Report No. 75. Experiment P-90-4, (e) Report No. 80. Experiment P-89-28, (f) Report No. 83,. Experiment A-88-20.

Goodale, W.S. (1988). American Cyanamid Company Report FD 36:: (a) Report No. 122 . Experiment B-88-12A, (b) Report No. 124,. Experiment B-88-14A, (c) Report FD 36: Report No. 125. Experiment B-88-14.

Guzman, B. (1990). American Cyanamid Company Method M 2023; "Chlortetracycline (CL 13,555).

Guzman, B (1990c). American Cyanamid Company Report C 3455; "Chlortetracycline (CL 13,555):

Guzman, B. (1993). American Cyanamid Company Method M 2328; "CL 13,555 (Chlortetracycline).

Guzman, B. (1991). American Cyanamid Company Report C 3474; "Chlortetracycline" (CL 13,555).

Henderson Jr, B.W., J.W. Cobble and H.L. Easterbrooks. (1953). *J Dairy Sci*, 40, 617.

Hewell, D.B. (1967). American Cyanamid Company Report B-428. Experiment L-1232.

Kanegis, L. (1958). Antibiotic A-VIII. The comparative pharmacology of the tetracyclines, American Cyanamid Company Report P.R. 4: 884-921.

Katz, S.E., Fassbender, C. A. and Dowling, J. J. Jr. (1972). *J Assoc Off Anal Chem*, 55, 128-133.

Kelly, R.G. (1964). American Cyanamid Company Report P.R. 9: 485-492. (Pearl River).

Kilroy, C.R., Hall, W.F., Bane, D.P., Bevill, R.F. and Koritz, G.D. (1990). *J vet Pharmacol Therap*, 13,: 49-58.

Korsrud, G.O. and J. D. MacNeil. (1987). *Food Additives and Contaminants*, 5, 149-153.

Langner, P.H. (1976). American Cyanamid Company Report FD 24: 41-129. Experiment B-75-17-FT.

Luthman, J. and Jacobsson, S-O. (1983). *Nord Vet-Med*, 35,: 292-299.

MacNeil, J.D., Martz, V.K., Korsrud, G.O., Salisbury, C.D.C., Oka, H., Epstein, R.L., and Barnes, C.J., (1996). Chlortetracycline, oxytetracycline and tetracycline in edible animal tissues, liquid chromatographic method: Collaborative study. *J Assoc Off Anal Chem International*, 19, 405-417.

Mariano, M. (1991). American Cyanamid Company Method M 1870.02;

Mariano, M. (1989). American Cyanamid Company (a) Method M 1868.1; (b) Method M 1869.1.

Meredith, W.E., H.H. Weiser and A.R. Winter. (1965). *Applied Microbiology*, 13: 86-88.

Miller, B.L. and Wyatt, R.D. (1985). *Poultry Sci*, 64, 1637-1643.

Munger, L.L. (1978). American Cyanamid Company Report FD 26: I066-1108. Experiment B-78-1.

Nelson, J. (1968). Cyanamid International Report.III: Report No. 32.

Neuschl, J. (1991). *Arch Exp Veterinarmed*, 45, 105-112.

Oka, H., Matsumoto, H., Uno, K., Harada, K.-I., Kadowaki, S. and Suzuki, M. (1985). Journal of Chromatography. 325, 265-

Oka, H. and Paterson, J. (1995). Chemical Analysis of Tetracycline Antibiotics. in 'Chemical Analysis of Antibiotics Used in Agriculture' Oka, H., Nakagawa, H., Harada, K.I., MacNeil, J. D. (eds), AOAC International, Arlington, VA.

Oka, M., Ikai, Y., Mayakawa, J., Harada, K.-I., Suzuki, M., Himei, R., Horie, M., Nakazawa, K.-I., Suzuki, M., Humei, R., Horie, M., Nakazawa, H. and MacNeil, J.D. (1993). *J Agric Food Chemistry*, submitted for publication.

Pollet, R.A., Glatz, C.E., Dyer, D.C. and Barnes, H.J. (1983). *Am J Vet Res*, 44, 1718-1721.

Pollet, R.A., Glatz, C.E. and Dyer D.C. (1984). *Poultry Sci*, 63, 1110-1114.

Rooney, K.A. (1988). American Cyanamid Company Report FD 36: (a) Report No. 120. Experiment B-88-l0A, (b)Report No. 121. Experiment B-88-10.

Rooney, K.A. (1989). American Cyanamid Company Report FD 37: (a) Report No. 2. Experiment B-88-l5A, (b) Report No. 11. Experiment B-88-15, (c) Report No. 14. Experiment P-88-14A., (d) Report No. 19 Experiment P-88-14

Rooney, K.A. (1990). American Cyanamid Company Report FD 38: Report No. 22. Experiment P-897.

Roudaut, B., Moretain, J.P. and Boisseau J. (1989). *Food Additives and Contaminants*, 6:, 71-78.

Sande, M.A., and Mandell, G.L. (1990). Antimicrobial Agents. Tetracyclines, Chloramphenicol, Erythromycine and Miscellaneous Antibacterial Agents. in Goodman and Gillmans's The Pharmacological Basis of Therapeutics, 8th Edition, Gilman, A.G., Rall, T.W., Nies, A.S. and Taylor. P. *eds*.p 1117-1145.

Sass, B., and Messersmith, R. (1964). American Cyanamid Company Report FD 12: 790-815. Experiment P-64-14-FT.

Schumacher, W.E. (1968). American Cyanamid Company Report FD 16: 352-378. Experiment A-67-36-FT.

Shor, A.L. (1961b). American Cyanamid Company Report C.F. 5: 115-120. Experiment BD-61-1-LT.

Shor, A.L. (1963). American Cyanamid Company Report CD II: 281-289. Experiment A-63-4-FT.

Shor. A.L. and G.O. Gale. (1965). American Cyanamid Company Report FD 13: 387-405. Experiment A-64-4-FT.

Shor, A.L., Johnson, W.P. and Abbey A. (1959). *J Dairy Sci*, 42, 1203-1208.

Stoner, J. (1962b). American Cyanamid Company Report CD I: 137-150. Experiment P-61-1-LT.

Stoner, J.C. (1963). American Cyanamid Company Report CD II: 1-64. Experiment A-62-14-LT

Sutter, H.M. and Wanner, M. (1990). *Tierarztl Prax*, 18, 27-32.

Tindall, E.E. and Berger, H. (1966a). American Cyanamid Company Report FD 14: (a) 976-1001. Experiment A-66-4-FT, (b) 1170-1213. Experiment A-65-30-FT, (c) 1214-1261. Experiment A-65-38-FT, (d) 1323-1381. Experiment A-65-37-FT.

Wahlstrom, R.C., Balios, J.P., Baker, R. and Libal, G.W. (1982). *Nutr Rep Int*, **26**, 873-878.

Wang, G.T. (1971). American Cyanamid Company Report **FD 19: (a)**: 1309-1371.

Wulf, R.J. and Eisner, H.J. (1961). American Cyanamid Company Report **C.P. 1**; 626-683. (Pearl River).

CLENBUTEROL

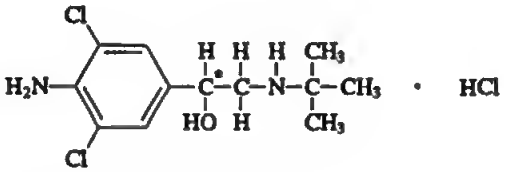
First draft prepared by
Dr. Raymond J. Heitzman
Compton, Newbury
Berkshire, United Kingdom

IDENTITY

Chemical name: 4-Amino-alpha-[(tert-butylamino)methyl]-3,5-dichlorobenzyl alcohol hydrochloride (IUPAC)

CAS number: 21898-19-1 (hydrochloride); 37148-27-9 (clenbuterol)

Structural formula:



Molecular formula: $C_{12}H_{19}N_2OCl_3$ (as hydrochloride)

Molecular weight: 313.65

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Appearance: Colourless microcrystalline powder (Merck Index)
White or slightly yellowish substance (sponsor)

Melting point: 174-175.5°C (Merck Index)
170-176°C (sponsor)

Solubility: Very soluble in water, methanol and ethanol, slightly soluble in chloroform, insoluble in benzene (Merck Index)

Soluble in water, methanol and ethanol, very soluble in chloroform (sponsor)

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Clenbuterol is used as a bronchodilator for horses and non-lactating cattle. The recommended treatment schedule is 0.8 μg/kg BW twice daily. The maximum duration of treatment in non-lactating cattle is 10 days. It may be administered by the oral or intravenous routes of administration. Cattle may also be injected by the intramuscular route.

Clenbuterol is also used as a tocolytic in cattle. The recommended treatment schedule is a single parenteral injection equivalent to 0.8 μg/kg BW.

METABOLISM

Pharmacokinetics

The radiolabel used in all of the studies in this monograph was ^{14}C at the C-2 position. The radiochemical purity was >95%.

Plasma

Clenbuterol was well absorbed after oral administration to laboratory animals, humans and the target species. In rats (Kopitar & Zimmer, 1973), dogs, rabbits (Zimmer 1974b) and humans peak blood concentrations were achieved 1-4 hours after oral dosing (Zimmer, 1976). Absorption was slower in another study in the dog (Zimmer, 1974a) and baboon (Johnston & Jenner, 1976) with peak plasma radioactivity occurring 6-7 hours after oral administration.

Peak plasma concentrations (range 0.24-1.8 μg/l) occurred in 0.25-3 hours following i.m. administration to calves or cows except in one study in three cows where the maximum concentration occurred at 8 hours; the time to peak concentration was 6-12 hours after oral administration (see text, Table 1, LLoyd-Evans, 1994). The plasma half-life in cattle varied from 16 to 105 hours depending on the subpopulation tested. Peak plasma concentrations (range 0.37-1.59 μg/l) occurred in 1.5-3 hours following i.m. or oral administration to horses with half-lives in the range 9-21.4 hours (Hawkins et al, 1984, Johnston & Dunsire, 1993).

Excretion into Faeces and Urine

Laboratory Animals and Horses

After oral administration of ^{14}C-Clenbuterol the radioactivity was quickly distributed throughout the tissues of rats and mice and shown to cross the placental barrier of the mouse (Kopitar 1969), the dog (0.43% of dose in 4h) (Rominger & Schrank, 1982) and the baboon (1.5% of dose in 3.5 h) (Schmid, 1980). The excretion of ^{14}C-Clenbuterol after oral administration is summarised in Table 1. The results indicate that the major fraction of the drug is excreted into the urine. Similar patterns of excretion were observed if the drug was administered parenterally or by inhalation (Huntingdon Res. Centre, 1978).

Table 1. Excretion of ^{14}C-Clenbuterol after oral administration

Species	Time period after dose (h)	% dose in urine	% dose in faeces	Reference
Rat	0 - 72	62.5	20.8	Kopitar, 1970
Rabbit	0 - 72 0 - 96	88.5 92	8.9 0.2 - 5	Zimmer, 1971 Zimmer, 1974b
Dog	0 - 96 0 - 96	85 - 87 74	3.5 - 9 3.7	Zimmer, 1974a Zimmer, 1974b
Horse	0 - 336	75 - 91	6 - 15	Johnston & Dunsire, 1993
Baboon	0 - 120	62*	16	Johnston & Jenner, 1976

* includes cage washings but not cage debris.

Cattle

Eight studies (Nos. 1-8 in Table 2) using cattle administered ^{14}C-clenbuterol either orally, as an intramuscular or intravenous injection, showed that excretion as a percentage of the dose was 50 - 85% in the urine, 5 - 30% in the faeces and where applicable, 0.9 - 3% in the milk when measured both during the dosing period and for 4 - 15 days after dosing.

Table 2. Studies using ^{14}C-Clenbuterol in cattle and equines

Study #	Animals	Dose regime	Tissues	Sampling times		Reference
				P.U.Fc. (h[A])	M.L.K.F.IS (days[B])	
1	9 preruminant calves	0.8 b.i.d 11 im x 4 oral x 7	P.U.Fc.M.L.K.F.IS	0-360 (3)	1, 7, 10	Hawkins et al, 1985b
2	9 ruminant calves	0.8 b.i.d 21 im	P.M.L.K.F.IS	0- (3)	0.25, 6, 10	Cameron et al, 1987
3	12 ruminant calves	0.8 b.i.d 21 im	P.M.L.K.F.IS		0.25, 5, 28	Hawkins et al, 1993b
4	3 cows	0.8 b.i.d 11 im x 6 oral x 5	P.U.Fc.Mk.M.L.K.F.IS	0-240	2h, 2, 5	Hawkins et al, 1985a
5	1 cow	1.6 single oral 0.8 s.i.d. oral x 3	P.U.Fc.Mk.	0-240		Schmid & Zimmer, 1977a
6	1 cow	0.8 single im 0.5 s.i.d. im x 3	P.U.Fc.Mk.	0-238		Schmid & Zimmer, 1977b
7	3 cows 3 cows 3 cows 9 cows	0.8 single oral 0.8 single iv 0.8 single im 0.8 single im	P.U.Fc.Mk. P.U.Fc.Mk. P.U.Fc.Mk. P.U.Fc.Mk.M.L.K.F.IS	0-144 0-144 0-144 0-144	 0.25, 3, 6	Cameron & Phillips, 1987
8	1 cow	0.6 single iv	P.U.Fc	0-96		Schmid, 1977
9	3 cows	0.52-0.74 s.i.d.	M.L.K.F.IS		0.5h, 3, 6	Schmid & Zimmer, 1977c
10	3 horses	0.8 b.i.d 21 oral	P.U.Fc. M.L.K.F.	0-396	1, 4, 6	Hawkins et al, 1984
11	12 horses	0.8 b.i.d 21 oral	P.U.Fc. M.L.K.F.	0-540	0.5,9,12,28	Johnston &Dunsire, 1993

Key; P, plasma; U, urine; Fc, faeces; Mk, milk; M, muscle; L, liver; K, kidney; F, fat; IS, injection site; s.i.d, once a day; b.i.d., twice daily; [A]sampling time from first dose; [B]sampling time from last dose; Key for dose regime: 0.8 b.i.d 11 im x 4 oral x 7 (Study 1) means 0.8 μg/kg bw twice daily im for 2 days (4 im doses then 0.8 μg/kg bw twice daily orally for 3 days + 0.8 μg/kg bw once a day for 1 day (7 oral doses) - total 11 doses of 0.8 μg/kg bw

Metabolism in laboratory animals

Clenbuterol was the major compound excreted in the urine of all the laboratory species examined. There was greater amount of metabolism in the rat compared to the other species tested. The contribution of Clenbuterol to the total residues found in urine after the administration of ^{14}C-Clenbuterol to several species is shown in Table 3.

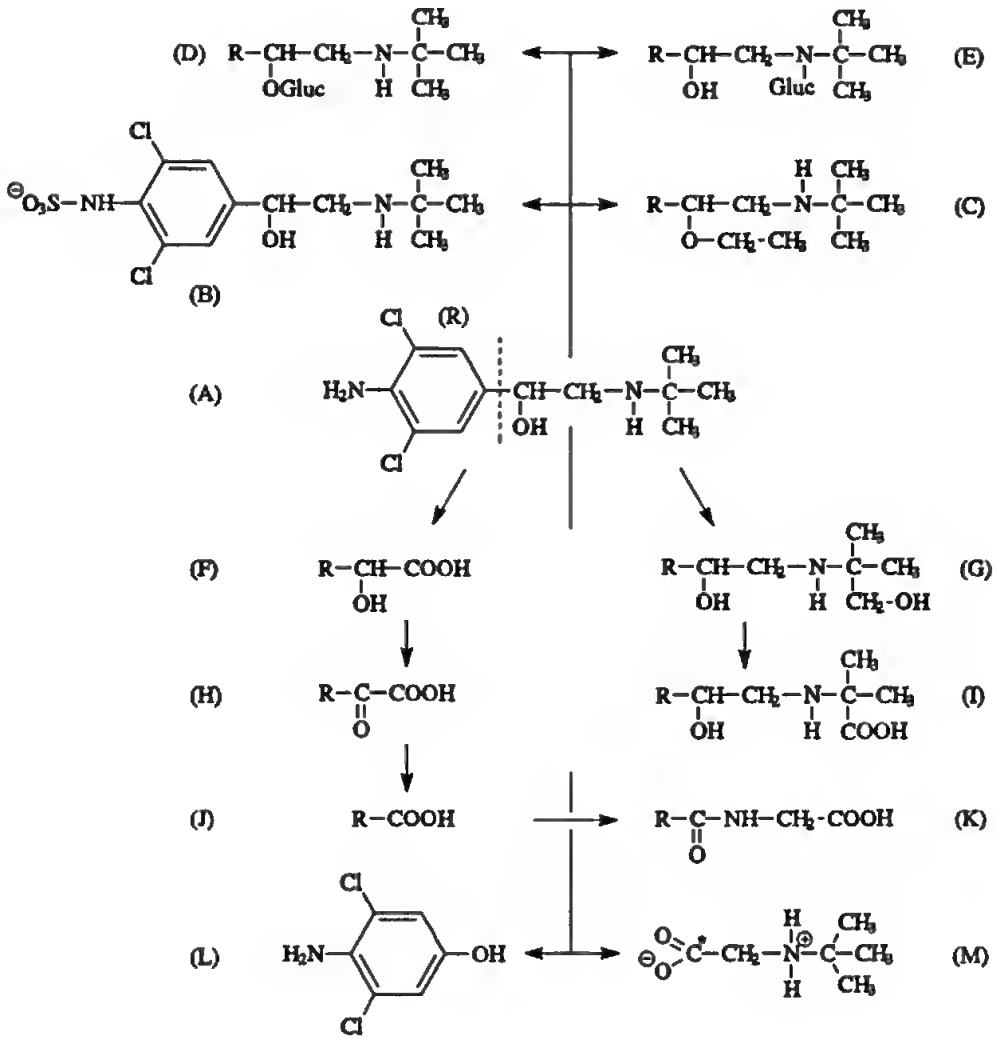
Table 3. Excretion of residues into the urine

Species.	Dose route	TR as % dose	CL as % dose	CL as % in TR	Reference
Rat	oral	43-58	32-42	ca. 73	Zimmer, 1971
Rabbit	oral	88	19	22	Zimmer, 1971
Rabbit	oral	89	34	38	Zimmer, 1974b
Dog	oral	66-107	17-20	ca. 20	Zimmer, 1974a
Dog	oral	72	15	21	Zimmer, 1974b
Baboon	oral	73	18	25	Johnston & Jenner, 1976
Baboon	i.v.	70	25	36	Schmid, 1982
Monkey	i.v.	48	n.m	n.m.	HRC, 1978
Man	oral	67	35	52	Zimmer, 1974c
Calf	i.m./oral	59-66	24-26	ca. 40	Hawkins et al., 1985b
	i.m.	n.m.	n.m.	42	Hawkins et al., 1993b
Cow	i.m./oral	47-67	22-49	n.m.	Hawkins et al., 1985a
Horse	oral	74		31-49	Hawkins et al., 1984

CL is Clenbuterol, TR is total residues, n.m. is not measured, i.v. is intravenous.

The metabolism of Clenbuterol was studied in more detail in the dog and the metabolic profile in the urine was determined (Schmid & Prox, 1986). The results are shown diagrammatically in Figure 1. The authors concluded that the biotransformation of Clenbuterol is slow (relative to other ß-agonists), since there are no direct points of access for the enzymes, monoamine oxidase and catechol-O-methyl transferase, or for efficient sulphate conjugation. The main metabolites were formed by oxidation along the long side chain in the 1 position of the ring, while the 2-amino-3,5-dichloro moiety remains intact.

Figure 1. Metabolic Profile of Clenbuterol in Dog Urine



(A) Clenbuterol (N-AB 365 Cl); (B) 2.3%; (C) 2.2%; (D) 0.5%; (E) 4.5%; (F) 20% 4-Amino-3,5-dichloromandelic acid (N-AB 739); (G) 1.6% NA 1141; (H) 6.5%; (I) 2.2%; (J) 9% 4-Amino-3,5-dichlorobenzoic acid (N-AB 930); (K) 19% 4-Amino-3,5-dichlorohippuric acid (N-AB 933); (L) -; (M) 2.2%.

Metabolism in Cattle

The metabolite profile seen in cattle is qualitatively similar to that seen in laboratory animals and in humans. Clenbuterol accounts for 60-86% and 22-53% of the total radioactivity in plasma (Schmid & Bucheler, 1987) and urine (Hawkins et al., 1985b) respectively. Other metabolites quantified in urine included N-AB 930 (ca. 3%), N-AB 931 (R-CHO)(2-4%), N-AB 933 (6-40%) and NA1141 (3-34%) (see Figure 1 for key to metabolites) (Hawkins et al, 1985b, Hawkins et al., 1993a, Hawkins et al., 1985a).

Metabolism of radiolabeled Clenbuterol in bovine liver followed a similar pattern with the majority of the extractable residues being Clenbuterol. The resulting profiles from several studies are shown in Table 4. The percentage of the total residues (%TR) which are extractable from the liver was >80% in livers collected 2-6 bours after drug administration. At longer withdrawal times (WT) the extractable fraction of the TR varied from about 50% in two studies to 87% in another study (see Table 4).

Table 4. Metabolic profiles of residues in bovine liver

Reference	WT (h)	TR extractable (%)	as a % extractable TR					
			NAB 365 (UD)	NAB 930	NAB 931	NAB 933	NA 1141	Polar/base -line
Hawkins et al, 1985b	24	50	64	ND	ND	ND	ND	26
Hawkins et al, 1985a	2	89	65	ND	ND	ND	ND	35
Baillie et al., 1980	3	89	52*	ND	ND	4	11	34
Hawkins et al, 1993b	6	81	90	0.3	1.4	1.5	2.1	4
	120	87	49	4.7	ND	4.8	ND	42
Cameron & Phillips, 1987	6	84	95	ND	ND	ND	ND	5

See Figure 1 for key to metabolites. UD is the unchanged drug, clenbuterol.
* NAB 365 Cl and NAB 739 could not be further separated due to their having similar chromatographic properties.

The data in Table 4 are used to calculate the content of Clenbuterol as a percentage of the total residues and the results are given in the last column of Table 5 . There are differences in the values for the two methods. This is most noticeable for the value for 120 bours withdrawal time wbere the value of 14% by the GC-MS method may be low. However the proportion of Clenbuterol in horse liver samples taken at 9 or 12 days WT was <10% (Johnston & Dunsire, 1993; Hawkins et al, 1993c).

The metabolism of clenbuterol in bovine kidney is similar to the one described for liver (Hawkins et al, 1985a&b,1993a). Parent compound accounts for 58-85% of the extracted radioactivity at 6 hours post dose. In muscle and milk parent compound makes up for 70-100% of the total radioactivity (Schmid, 1990a&b).

The pharmacological activity of the metabolites was determined and only compound NA1141, with an activity of 20% that of Clenbuterol hydrochloride, possessed any activity.

Selected tissues from the radiodepletion studies were analysed by a GC-MS method for the content of Clenbuterol (Schmid, 1990b). The results are shown in Table 5.

Table 5. Clenbuterol measured by GC-MS and its percentage of total residues in bovine liver

Animals	WT (h)	Total Residues (µg/kg)	Clenbuterol (µg/kg)	Clenbuterol as % TR	
				GC-MS	Table 4
Calf	24	9.2	5.3	58	32
Calf	6 120	20.7 3.9	13.1 0.6	63 14	73 43
Cow Cow	2 48	29.8 8.6	27.2 4.4	91 51	58 45

Metabolism in the Horse

Clenbuterol accounts for 45% of the total radioactivity in plasma (Zimmer,1977). In urine, 45% of the excreted radioactivity is parent compound clenbuterol. The metabolite pattern in urine obtained during a repeat-dose residue study (Hawkins et al., 1984) showed that parent component accounted for 31-49% of urine radioactivity, NAB 821 (R-CHOH-CH_2OH) for 0-11% and NA 1141 for 10-16%. Approximately 23-30% of urine radioactivity remained at the baseline during metabolite profiling.

Investigations in equine liver tissue, the target tissue for ^{14}C-Clenbuterol, revealed that parent compound accounts for 38-90% of total radioactivity at early sacrifice time points of 12 and 24 hours post dose (Hawkins et al., 1984, Hawkins et al., 1993c). Extraction efficiency at these time points ranges between 59% and 85%. Apart from clenbuterol, NA 1141 (10%) and NAB 821 (3-7%) could be identified in equine liver tissue. At later sacrifice time points there was a quantitative change in the metabolite pattern observable, though not a qualitative one. The metabolism of clenbuterol in horse kidney is similar to the one described for liver. Parent compound accounts for 89% of the extracted radioactivity at 24 hours post dose, while NA 1141 makes up 11%.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Cattle

Residue depletion studies were performed in both calves and cows using ^{14}C-Clenbuterol administered by the i.m. and/or the oral routes. The studies used are those numbered in Table 2; 1,2 and 3 for calves and 4, 7 and 9 for cows. Depletion of total residues is rapid in all edible tissues of calves and cows (see Table 6 for references and results).

In calves, the results from GLP-certified total balance studies (No. 1,2) show that in individual calves killed at 6,7 or 10 days after last dose, total residues had fallen below limits of detection (0.06 µg/kg and 0.18 µg/kg, respectively) in muscle at all time points. Study 3, a GLP-certified study, uses adequate numbers of calves to establish that at 28 days, total residues in liver are less than 1 µg/kg and in muscle and at the last two injection sites less than 0.2 µg/kg. The calves in study 3 received the dose of clenbuterol hydrochloride for the maximum time as intended for the respiratory preparation, 10.5 days of twice-daily treatments at 0.8 µg/kg.

In cows there are three studies. Study 9 is an in-house orientation study in three cows; study 4, GLP-certified,

Table 6. Total residues (mean ± SD μg/kg) of radioactivity in tissues after administering ^{14}C-Clenbuterol to calves and cows

Study No.	No. cattle	WT (days)	Muscle	Liver	Kidney	Fat	Injection Site	Reference Table 2.
Calf	3	1	0.86 ± 0.39	9.20 ± 3.33	9.09 ± 3.74	0.96 ± 0.58	0.98 ± 0.33	Hawkins et al.
1	3	7	ND	1.39 ± 0.19	0.41 ± 0.02	ND	0.13 ± 0.16	1985b
	3	10	ND	0.85 ± 0.10	0.27 ± 0.07	ND	0.08 ± 0.10	
2	3	0.25	2.17 ± 0.27	36.6 ± 9.5	38.7 ± 8.4	0.82 ± 0.42	2.49 ± 0.70	Cameron et al.
	3	6	0.09 ± 0.10	7.37 ± 2.2	3.16 ± 0.5	ND	0.32 ± 0.20	1987
	3	10	ND	4.32 ± 0.5	2.15 ± 0.6	ND	0.28 ± 0.20	
3	4	0.25	0.79 ± 0.2	20.7 ± 4.8	16.1 ± 2.3	0.55 ± 0.1	1.66 ± 0.3	Hawkins et al.
	4	5	0.16 ± 0.03	3.9 ± 0.7	2.2 ± 0.5	0.12 ± 0.2	0.39 ± 0.1	1993b
	4	28	ND	0.89 ± 0.1	0.46 ± 0.2	ND	0.18 ± 0.03	
Cows	1	2 hours	1.45	29.8	14.7	0.58	4.79	Hawkins et al.
4	1	2	0.34	8.6	3.9	0.31	3.24	1985a
	1	5	0.19	4.4	1.4	0.35	2.92	
7	3	0.25	0.22 ± 0.14	6.26 ± 0.92	5.11 ± 1.27	0.09 ± 0.13	5.39 ± 4.13	Cameron &
	3	3	0.01 ± 0.01	1.17 ± 0.47	0.42 ± 0.13	0.02 ± 0.04	0.14 ± 0.24	Phillips
	3	6	0.01 ± 0.01	0.65 ± 0.24	0.18 ± 0.09	0.02 ± 0.04	0.22 ± 0.32	1987
9	1	0.5 hours	0.67	8.19	8.06	0.23	130.6	Schmid &
	1	3	0.03	1.96	0.99	0.07	0.31	Zimmer
	1	6	ND	0.84	0.2	0.08	2.53	1977c

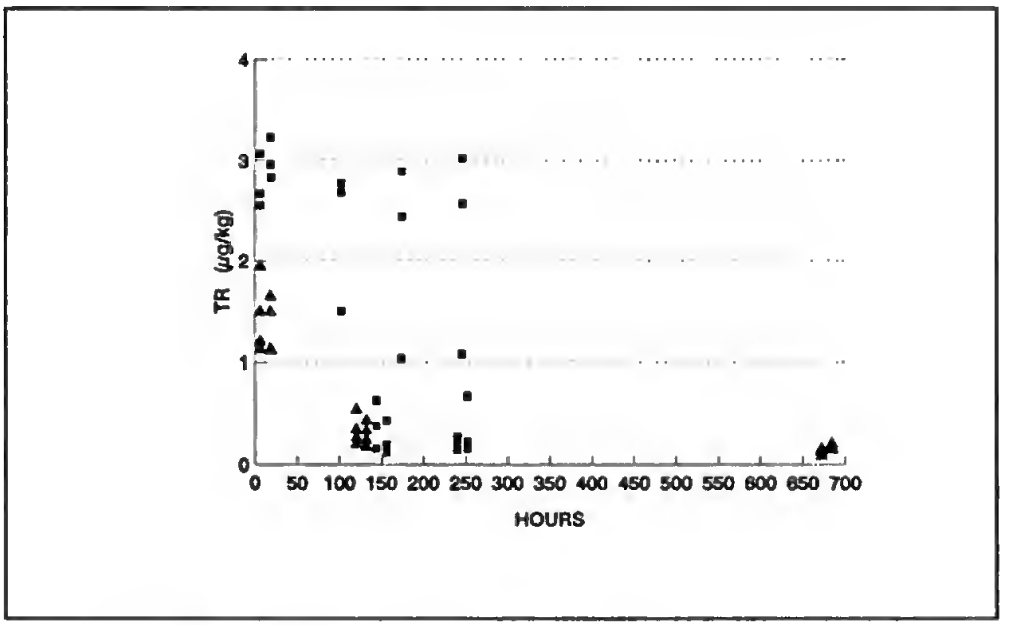
The dosages are given for each study and referenced in Table 2. ND is not detected with LOD Study 1, 0.06 μg/kg muscle; 0.17 μg/kg fat; Study 2, 0.18 μg/kg; Study 3, 0.1 μg/kg.

concerns total balance following repeated doses of clenbuterol hydrochloride. These demonstrate rapid depletion of radioactivity from edible tissue. Study 7, GLP-certified, confirms this using a total of 9 lactating dairy cows which received the recommended single injection of clenbuterol hydrochloride, as intended for the tocolytic preparation. By 6 days, total residues in liver were less than 1 μg/kg, in muscle less than 0.1 μg/kg, and in samples of injection site were less than 0.5 μg/kg.

Total Residues at the Injection Site

In several of the studies in which Clenbuterol is administered intramusculary there were both single and multiple injections. In one study in calves, 21 injections of 0.8 μg/kg BW were given at different sites over a period of 10.5 days (Cameron et al., 1987). Samples were collected from the multiple sites and analysed for total residues (radioactivity). Because the injections were given at different times data for a wide range of times after dosing is possible even in the same calf. The results are plotted in figure 2 for time after injection versus the residue. There is a wide variation in the results and there is no correlation between time after dosing and the concentration of residues at the injection sites, in fact the residues found at 246 hours were as high as those seen at 6 hours after injection. The same sampling schedule was not followed in a later study (Hawkins et al., 1993b) where only samples of injection sites were collected from the sites of the last two injections in each calf, i.e. injected on days 10 and 11.

Figure 2. Radioactive residues at intramuscular injection sites of calves



Data (■) from Cameron & al, 1987; (▲) Hawkins et al, 1993b.

Residues in Milk

In a GLP study using 9 Friesian cows, mean body weight 538 kg, the cattle were divided into groups of 3 and given a single dose of 0.8 μg/kg BW [^{14}C]-labelled clenbuterol hydrochloride by the oral, intravenous or intramuscular route. Samples of milk were taken for analysis (Cameron & Phillips, 1987) and the results are shown in Table 7.

Table 7. Total residues in milk after administration of ^{14}C-Clenbuterol by different routes

Withdrawal Intervals (h)	Total Residues (μg eq/l) in milk from groups of three (3) cows								
	Oral			Intravenous			Intramuscular		
Pre-dosing	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	0.40	0.42	0.37	0.62	0.72	0.58	0.53	0.84	0.68
23	0.51	0.43	0.35	0.27	0.29	0.37	0.31	0.51	0.36
31	0.40	0.34	0.28	0.16	0.16	0.27	0.19	0.35	0.24
47	0.21	0.15	0.12	0.08	0.08	0.15	0.09	0.16	0.10
55	0.14	0.11	0.10	0.06	0.05	0.11	0.07	0.12	0.07
71	0.08	0.06	0.04	0.07	0.03	0.07	0.04	0.07	0.04
79	0.06	0.04	0.02*	0.02*	0.02*	0.06	0.03	0.06	0.03
95	0.03	0.03	0.01*	0.01*	0.01*	0.03	0.02*	0.04	0.02*
103	0.03*	0.01*	0.01*	0.02*	0.02*	0.02*	0.02*	0.03	0.01*
119	0.02*	0.01*	0.01*	0.01*	0.01*	0.02*	0.01*	0.02*	0.02*

* derived from data <30 dpm above background; ND is not detected and derived from data <10 dpm above background

In a second study all 9 cattle were given a single dose of 0.8 μg/kg BW [^{14}C]-labelled clenbuterol hydrochloride by the intramuscular route (Cameron & Phillips, 1987). Three cows were slaughtered for tissue residue analysis before the milk was collected and the residues in the milk for the remaining 6 cows were measured. The results are shown in Table 8.

In a non-GLP study these milk samples were analysed by GC-MS to determine the residues of clenbuterol (Schmid 1990a). The results for the total residues and clenbuterol are summarised in Table 8. For the first 2-3 days after the end of treatment, most of the residues in milk consisted of unmetabolised clenbuterol. The very low concentrations (0.015-0.050 μg/l at 79 hours post injection) of radioactivity at subsequent time points did not appear to be clenbuterol. [Note: The LOQ claimed for the method is 0.050 μg/l but this is not substantiated in Schmid, 1990a]

In a GLP study, 3 lactating cows were given twice daily i.m. doses of the combination product ^{14}C-clenbuterol (0.8 μg/kg BW)/sulphadiazine (12.5 mg/kg BW)/trimethoprim (2.5 mg/kg BW) for 3 consecutive days (Hawkins et al 1985a). They were then given twice daily oral doses on days 4 - 5 and a single oral dose on day 6. The total residues in the milk reached peak values of 3.2, 3.5 and 3.9 μg/l during administration and had declined to 0.18 μg/l by 108 hours post-dosing in the one cow kept on the study. The residues in milk of this cow collected at 12 hour intervals (twice a day milkings) after the last dose were: 1.58, 1.04, 0.77, 0.51, 0.39, 0.27, 0.21 and 0.18 μg/l. These high and persistent residues in milk clearly support the contraindication for this particular therapeutic use in lactating cows.

Table 8. The residues (μg/l) in milk of radiolabeled clenbuterol as total residues (TR) and clenbuterol (CL)

Cow No.	Time in hours after i.m. injection													
	7	7	23	23	31	31	47	47	55	55	71	71	79	79
	CL	TR	CL	TR	CL	TR	CL	TR	CL	TR	CL	TR	CL	TR
4			0.230	0.300			0.056	0.090	0.031	0.070	0	0.040		
5			0.259	0.330			0.090	0.110	0.061	0.080	0	0.040		
6			0.392	0.330			0.107	0.130	0.056	0.090	0.019	0.060		
7	0.634	0.750	0.368	0.330	0.167	0.210	0.084	0.100	0.039	0.070	0	0.040	0	0.030
8	1.318	0.930	0.426	0.430	0.272	0.330	0.127	0.160	0.094	0.120	0.031	0.070	0	0.050
9	0.691	0.650	0.272	0.340	0.269	0.260	0.057	0.090	0.030	0.060	0	0.030	0	0.020
Mean	0.881	0.777	0.325	0.343	0.236	0.267	0.087	0.113	0.056	0.075	0.008	0.047	0	0.033
SD	0.380	0.142	0.081	0.045	0.060	0.060	0.028	0.027	0.022	0.030	0.013	0.015	0	0.015
% CL of TR	113%		95%		88%		77%		75%		17%		0%	

The cows were administered an i.m. injection of ^{14}C-Clenbuterol at a dose of 0.8 μg/kg BW and approximately 2500 dpm/kg BW (Schmid, 1990a).

Horse

Residues in the edible tissues were determined in three horses receiving oral doses of a formulation combining clenbuterol hydrochloride with two antibiotics. The animals were treated twice daily for ten days and then a final oral dose on day 11 (see Study 10 Table 2). A similar study, however, applying only clenbuterol hydrochloride, was carried out using 12 horses and administering 21 oral doses (see Study 11 Table 2). The results are shown in Table 9. The total residues were highest in liver and kidney, very low in muscle and not detectable in fat.

Table 9. Total residues of radiolabeled compounds (μg/kg clenbuterol equivalents)

Withdrawal time (days)	Muscle	Liver	Kidney	Fat	
Study 10					
1	0.21	11.27	3.43	<0.17*	
4	<0.17*	3.09	0.43	<0.17*	
6	0.29	3.30	0.23	<0.17*	
Study 11				RF	OF
0.5	0.35	16.71	4.20	<0.35*	<0.00*
9	0.00*	5.55	0.35	<0.06*	<0.07*
12	0.01*	4.54	0.24	<0.00*	<0.00*
28	0.01*	0.65	0.18*	<0.09*	<0.02*

*values below limit of quantification (<10 dpm above background); RF is renal fat; OF is omental fat.

Other Residue Depletion Studies (with unlabeled drug)

Stability of Residues

The effect of cooking on the beat stability of clenbuterol was investigated (Rose et al. 1995). The drug was stable in boiling water at 100°C. In cooking oil at 260°C losses were observed, indicating a half-life of about 5 min. The effect of a range of cooking processes (boiling, roasting, frying, microwaving) on clenbuterol residues in fortified and incurred tissue was studied. No net change in the amount of clenbuterol was observed in any of the cooking processes investigated except for deep frying using extreme conditions. There was little observed migration from the tissue into the surrounding liquid or meat juices. Clenbuterol residues were found not to be evenly distributed in the incurred raw tissue used for the investigation. The findings of this investigation show that data obtained from measurements on raw tissue are applicable for use in consumer exposure estimates and dietary intake calculations.

Depletion Studies

There were no studies submitted by the sponsor but numerous studies are reported in the open literature. These include studies using the recommended therapeutic dosage and numerous studies in which clenbuterol was administered at a dose (ca. 10 times the therapeutic dose) to enhance the growth performance of farm animals. The general conclusions were that residues of unchanged clenbuterol accumulate in the eyes, lungs, hair and

feathers. The highest residues in the "basket" tissues were found in the liver and kidney. The residues of clenbuterol in tissues and body fluids were measured in cattle treated with the therapeutic dose of the drug (Elliott et al, 1995). During treatment many tissues and body fluids contained residues of clenbuterol. After a 14 day withdrawal period residues of clenbuterol were detectable only in the eyes (mean 27.1 μg/kg) and to a much lesser extent lung and kidney (mean 0.3 μg/kg). By day 28 residues were only detected in eyes (mean 6 μg/kg) and these were still present at day 42. The authors conclude that it is not possible to differentiate between the legal and illegal use of the drug solely on residue analysis. This is in contrast to the opinion of the sponsor who believes that differentiation between legal and illegal use would be possible based on liver analysis, if the analytically determined concentrations of clenbuterol were related to the withdrawal time claimed to have been observed by the farmer.

Seven female Brown Swiss calves were used to study the pharmacokinetics of clenbuterol after an effective anabolic dosage of 5 μg/kg BW was given twice daily for 3 weeks. (Meyer & Rinke, 1991). Analyses of clenbuterol concentrations in different tissues was done by enzyme immunoassay (EIA). Tissue samples were taken from three calves on the last day of administration and from two more after 3.5 or 14 d of clenbuterol withdrawal. The rate of clenbuterol elimination was dependent on time and tissue clenbuterol concentrations in the lung dropped from a mean of 76 μg/kg to a level of less than 0.8 μg/kg after 14 days whereas in the liver the clenbuterol concentrations decreased from 46 μg/kg to 0.6 μg/kg within 14 d of withdrawal. Highest levels were always found in the eye: 118 μg/kg, 57.5 μg/kg and 15.1 μg/kg after 0, 3.5 d and 14 d of withdrawal, respectively.

Bound Residues/Bioavailability

The majority of the radiolabeled residues were extractable with mild solvents. The amount of bound residues is small and insufficient to be taken into account in the calculation of MRLs.

METHODS OF ANALYSIS RESIDUES IN TISSUES AND MILK

There are more than one hundred methods, published in the open literature since 1990, for the determination of residues of clenbuterol and other similar ß-agonists in biological samples (for examples and full details of selected methods used in the EU, see Heitzman 1994). The methods for screening include EIA, HPLC and GCMS. Confirmation of positives is performed using specific GCMS methods with sensitivities for edible tissues from 0.01 μg/kg upwards and limits of quantification (determination) from 0.02 μg/kg upwards. A typical example is the GC-MS method described by Girault & Fourtillan (1990) who measured clenbuterol and the internal standard [$^2H_9$]-clenbuterol as the perfluoroacyl derivatives (m/z 368 and 377). Accuracy and precision were determined for fortified bovine tissue samples:

theoretical concentration (μg/kg)	no samples	mean observed concentration (μg/kg)	S.D.	CV (%)	Error (%)
0.200	10	0.213	15.6	7.3	+6.4
0.020	10	0.019	1.7	9.0	-5.5

The LOD was 0.010 μg/kg (based on the mean signal ± SD for 10 "blank" samples being significantly different ($p < 0.001$) from that for 0.010 μg/kg samples). This method was also validated for bovine and equine liver by Hawkins et al (1993a, 1994) with acceptable accuracy and precision at the LOQ of 0.100 μg/kg.

The method proposed by the sponsor is based on GC-MS. Samples of muscle and liver were prepared by maceration and digestion with enzymes (subtilisin) followed by extraction with reversed phase material (C-18 Sep-Pack), clean-up by solvent distribution and derivatisation (silylation). The ion m/z 351 was used for quantification (Schmid & Bucheler, 1987). A very similar method is described for milk (Schmid, 1990a). Specificity was demonstrated against matrix "blanks". It was shown that trimethoprim and sulfadiazine, which may be co-administered with clenbuterol, did not interfere with the assay. Clenbuterol metabolites have different lipophilicity, and molecular weights and so would not be expected to interfere. The LOQ was stated to be 0.100 μg/kg. However acceptable accuracy and precision were not demonstrated at this concentration and linearity was shown only over the range

0.250-2 μg/kg. The method was adapted for measuring residues in milk (Schmid, 1990a). Linearity for clenbuterol was achieved over the range 0.125-1 μg/l with recoveries of ^{14}C-clenbuterol of 77-106%. The claimed LOQ is 0.050 μg/l but no data is presented to support this.

APPRAISAL

Clenbuterol is manufactured as a 50:50 racemic mixture. Most of the pharmacological activity is associated with the levo form. It is a direct-acting $ß_2$-sympathomimetic agent used to treat respiratory diseases in cattle and horses and is administered as multiple oral or parenteral doses. For noo-lactating cattle the maximum duration of treatment is restricted to 10 days. It is also used as a tocolytic in cattle when the recommended treatment schedule is a single parenteral injection equivalent to 0.8 μg/kg body weight. Although unapproved for such purposes it is used at doses many fold higher than the recommended therapeutic uses acting as a repartitioning agent in many farm species.

All the residue studies submitted by the sponsor were carried out using the ^{14}C-radiolabeled racemic (chiral) mixture and were compliant with GLP requirements. Clenbuterol was well absorbed after oral administration to laboratory animals, humans and the target species. In most species peak blood concentrations were achieved 2-3 hours after oral dosing. The plasma half-life in cattle varied from 16 to 105 hours depending on the sub-population tested. The substance was widely distributed in the tissues and was shown to cross the placenta in pregnant rats, dogs, baboons and cows. In all species, excretion was predominantly via the urine as unmetabolised clenbuterol.

Eight studies using cattle that were administered ^{14}C-clenbuterol either orally, or an intramuscular or intravenous injection, showed that excretion as a percentage of the dose was 50-85% in the urine, 5-30% in the faeces and where applicable, 0.9-3% in the milk when measured between administration and 4-15 days after dosing. After oral administration of radiolabeled drug to horses, 75-91% and 6-15% of the dose was excreted in the urine and faeces, respectively, over a 14 day period. The metabolic pathways were similar in all the species studied though there were quantitative differences in the amounts of metabolites formed.

The metabolite profile seen in cattle is qualitatively similar to that seen in laboratory animals and in humans. Metabolism of radiolabeled Clenbuterol in bovine liver followed a similar pattern with the majority of the extractable residues (>50%) being Clenbuterol. There were 4 minor metabolites and some unidentified polar metabolites.

Investigations in equine liver tissue using ^{14}C-Clenbuterol revealed that parent compound accounts for 38-90% of total radioactivity at early sacrifice time points of 12 and 24 hours post dose. Apart from clenbuterol one metabolite formed by hydroxylation of a tertiary methyl group (NA 1141)(10%) and NAB 821 (R-CHOH-CH_2OH)(3-7%) could be identified in equine liver tissue. At later sacrifice time points (>24 h) there was a quantitative change in the metabolite pattern observable, though not a qualitative one. The metabolism of clenbuterol in horse kidney is similar to the one described for liver. Parent compound in kidney accounts for 89% of the extracted radioactivity at 24 hours post dose, while NA 1141 makes up 11%.

In cattle the total residues were much higher in those receiving multiple daily doses compared with those administered a single injection. In both type of treatments the highest residues were observed in liver and kidney and very low residues were present in muscle and fat. The total residues were <0.3 μg/kg in muscle and fat from about 6 days after treatment with multiple doses but were at levels between 9.85 and 0.35 μg/kg in liver and kidney for 6 to 28 days post dosing. Residues in muscle and fat consisted mostly of clenbuterol shortly after administration but in liver and kidney the percentage declined with increasing withdrawal time. The relationship between residues of clenbuterol and the total residues was determined 6 hours, 3 days and 6 days after treatment. Residues in muscle (including injection site muscle) consisted mostly of clenbuterol. At the 6-hour time point, residues in liver consisted almost entirely of clenbuterol; after 6 days the percentage of clenbuterol had declined to less than 50%.

Residues at the injection sites in muscle varied and there was no correlation between the concentration and time during the first eleven days after dosing. However, in one study the residues were low (<0.25 μg/kg) at 28 days

after dosing.

The use of the drug as a tocolytic may result in residues in milk in the period following parturition. Potential levels of residues in milk as a result of this treatment were investigated by administering lactating cows with a single intramuscular (im) injection of radiolabeled clenbuterol. The residues in milk over a three day sampling period consisted almost entirely of unmetabolised clenbuterol. The very low concentrations, 0.015-0.050 μg/l at 79 hours post injection, of radioactivity at subsequent time points did not appear to be clenbuterol. In consideration of whether the drug could be administered to lactating cows in a multiple dosing formulation containing clenbuterol with two antibiotics, three cows were given i.m. injections followed by twice daily oral doses of radiolabeled Clenbuterol. The total residues in the milk reached peak values of 3.2, 3.5 and 3.9 μg/l during administration and had declined to 0.18 μg/l by 108 hours post-dosing in the one cow kept on the study. Because of these unacceptably high levels of residues this combination product is not recommended for use in lactating cattle.

Two studies were carried out in the horse in which the concentrations of total residues in tissues were compared with residues of unmetabolised clenbuterol. In both studies the pattern of residue depletion was similar to that of cattle. Three horses were dosed orally and the residues measured at 6 days withdrawal time were all less than 0.3 μg/kg in muscle, kidney and fat and greater than 3 μg/kg in liver. In a second study 12 ponies were dosed orally at the recommended dose level with radiolabeled drug and total residues were measured at 0.5, 3, 12, 28 days post dosing. At all times the total residues were <1 μg/kg in liver, <0.2 μg/kg in kidney and <0.1 μg/kg in muscle and fat.

There are more than one hundred methods published in the open literature since 1990 for the determination of residues of clenbuterol and other similar ß-agonists in biological samples. The methods for screening include EIA, HPLC and GCMS. Confirmation of positives is performed using specific GCMS methods with limits of detection (LOD) for edible tissues from 0.01 μg/kg upwards and limits of quantification (LOQ) from 0.02 μg/kg upwards. The sponsor's proposed routine analytical method was based on GC-MS. The LOQ was stated to be 0.10 μg/kg for tissues and 0.05 μg/l for milk but acceptable accuracy and precision had not been demonstrated at these concentrations. Another well validated method in the dossier, also based on GC-MS, had been shown to have a LOQ of 0.020 μg/kg and a LOD of 0.010 μg/kg for bovine tissues.

Maximum Residue Limits

The ADI of 0-0.004 μg/kg of body weight established by the Committee is equivalent to 0.240 μg per day for a 60 kg person. In recommending MRLs tbe Committee took account of the following factors;-

- Muscle and liver are the target tissues;

- The marker compound parent drug is the only residue of public health concern. Because the metabolites and bound residues are not of toxicological concern they may be discarded from the calculation of the MRL's;

- 100% of the total residues in muscle, fat and milk are unchanged drug and 60% of the total residues in bovine liver and kidney and 6% of the total residues in equine liver and kidney are unchanged drug;

- There are analytical methods suitable for regulatory use; and

- The sponsors are not proposing to make the drug available for multiple use in lactating cows.

The Committee recommends MRLs for cattle and borses of 0.2 μg/kg in muscle and fat, 0.6 μg/kg in liver and kidney, and of 0.05 μg/kg for cattle milk, expressed as parent drug. Using these values for the MRLs then the maximum theoretical intake for the food basket would be 0.235 μg (see Table 10).

Table 10. Intake of Clenbuterol at level of MRLs

Tissue	kg in basket	MRL (μg/kg)	μg
Muscle	0.300	0.2	0.060
Liver	0.100	0.6	0.060
Kidney	0.050	0.6	0.030
Fat	0.050	0.2	0.010
Milk	1.500	0.05	0.075
		Total	**0.235**

The Committee noted that the maximum residues observed at the recommended withdrawal times for single or multiple dose formulations when applied to the calculation of possible daily intake gives residues which are less than 0.130 μg in both cattle and horses (see Table 11).

Table 11. Estimation of residues of clenbuterol at practical withdrawal times for cattle and horses

Tissue	kg in basket	Cattle				Horses			
		Max CL at 6 d[S] (μg/kg)	Intake (μg)	Max CL at 28 d [M] (μg/kg)	Intake (μg)	Max CL at 12 d [M] (μg/kg)	Intake (μg)	Max CL at 28 d [M] (μg/kg)	Intake (μg)
Muscle	0.300	0.03	0.009	0.11	0.033	0.01	0.003	0.01	0.003
Liver	0.100	0.53*	0.053	0.62*	0.062	0.45**	0.045	0.05**	0.005
Kidney	0.050	0.17*	0.009	0.47*	0.012	0.03**	0.001	0.01**	0.001
Fat	0.050	0.12	0.006	0.11	0.006	0.00	0.00	0.23	0.012
Milk	1.500	0.01	0.015	0.01	0.015	NA	0	NA	0
		Total	0.092 μg	Total	0.128 μg	Total	0.049 μg	Total	0.021 μg

CL is clenbuterol; [M] is multiple treatments; [S] is a single injection; NA is not applicable; * value is 60% of total residues; ** value is 6% of total residues; (data for milk is limited to the use of a single i.m. injection as a tocolytic).

REFERENCES

Baillie, H.W. , Cameron, B.D., Draffan, G.H., Schmid, J., (1980). 13.11.80. Investigations of the placental transfer of ^{14}C-N-AB 365 CL in the cow. Report no. 111674 from Inveresk Research International ADME 21/80 - U-Venti 38, Plani 30, Venti TMP/S 30 - U80-0230. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Cameron, B.D., Phillips, M.W.A., Mikhail, M., (1987). 06.87. The residue kinetics of ^{14}C-N-AB 365 CL in the calf. IRI Project No. 135009 IRI Report No. 4371 Summary table on local tolerance*, 04.89 - U-Venti 83. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Cameron, B.D., Phillips, M.W A., (1987). 09.09.87. The residue kinetics of ^{14}C-N-AB 365 CL in the lactating cow. IRI Project No. 135014/IRI. Report No. 4445. Amendment to IRI Report No. 4445 of Hall, B.E. of 24.10.88 - U-Venti 90, Plani 56. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Elliott, C.T., McCaughey, W.J., Crooks, S.R.H., McEvoy, J.D.G. and Kennedy, D.G., (1995). Residues of clenbuterol in cattle receiving therapeutic doses: Implications for differentiating between legal and illegal use. Vet. Quarterly, 17, 100-102.

Girault, J., Fourtillan, J.B., (1990). Determination of clenbuterol in bovine plasma and tissues by gas chromatography-negative-ion chemical ionisation mass spectrometry. Biomed. Environ. Mass Spectrometry, 19, 80-88.

Hawkins, D.R., Cheng, K.N., Major, R.M., (1993a). 05.04.1993, Validation of a GC-MS method for the measurement of ^{14}C-Clenbuterol in calf liver and measurement of samples from calves administered with ^{14}C-Clenbuterol. (Study No. BOI/140) BOI 142/921659 - U-Venti 126, Plani 78-. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Hawkins, D.R., Cheng, K.N., Major, R.M., (1994). 14.02.1994. Bioanalytical report. Validation of a GC-MS method for the measurement of ^{14}C-Clenbuterol in horse liver and measurement of samples from horses administered with ^{14}C-Clenbuterol. Study No. IRI/150797 Sponsor's Study No. 6820 VEN 9212 BOI 150/942011 + Report Amendment dated 22.02.1994 -U-Venti 133, Venti TMP/S 109. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Hawkins, D.R., Elsom, L.F., de-Salis, C.M., Morris, G.R., Roberts, N.L., Cameron, D.M., Pugh, K.E., (1985a). 21.08.85. The disposition of the combination product 14-C-N-AB 365 CL/Trimethoprim/Sulfadiazine in dairy cows. Comment on the HRC report HRC / BOI III/84680A - U-Venti 76, Plani 47, Venti TMP/S 10. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Hawkins, D.R., Elsom, L.F., de-Salis, C.M., Morris, G.R., Roberts, N.L., Cameron, D.M., Offer, J., Fish, C., (1985b). 16.2.85. The disposition of the combination product 14-C-N-AB 365 CL Trimethoprim/Sulfadiazine in calves - U-Venti 70, Plani 42, Venti TMP/S 8-. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Hawkins, D.R., Elsom, L.F., de-Salis, C.M., Roberts, N.L., Cameron, D.M., (1984). 13.11.84. The disposition of the combination product ^{14}C-N-AB 365 CL/Trimethoprim/Sulfadiazine after oral administration to horses. With addenda: letter of K.E. Pugb to Dr. Hawkins of 22.08.85 and letter of Dr. Hawkins to K.E. Pugh of 09.09.85 - U-Venti 65, Plani 39, Venti TMP/S 7. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Hawkins, D.R., Elsom, L.F. , Dighton, M.H., Kaur A., Cameron, D.M., (1993b). 29.03.1993. The pharmacokinetics, metabolism and residues of ^{14}C-Clenbuterol (^{14}C-N-AB 365 CL) following intramuscular administration to calves. HRC/BOI 140/921418 - U-Venti 125. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Hawkins, D.R., Elsom, L.F . Dighton, M.H., (1993c). 15.10.1993. ^{14}C-N-AB 365 CL . Investigation of the metabolic profiles in the livers of the horses following multiple oral administration BOI 149/931490 -U-Venti 129, Venti TMP/S 105-. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Heitzman, R.J., (1994). Veterinary Drug Residues. Residues in Food Producing Animals: Reference Materials and Methods. 2nd Edition; EC Report EUR 15127. ECSC-EEC-EAEC, Brussels & Luxembourg, Publ. Blackwells Scientific. ISBN 0-632-03786-5.

Huntingdon Research Centre; (1978). 18.08.78. Pharmacokinetics of ^{14}C-N-AB 365 during chronic inhalation toxicity studies in cynomolgus monkeys - U79-0212 -. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Johnston, A.M., Dunsire, J.P., (1993). 29.04.1993. Plasma kinetics, metabolism, excretion and residue kinetics of N-AB 365 CL following multiple oral administration to the horse. IRI Project No. 150797, IRI Report No. 8346 - U-Venti 127, Venti TMP/S 104. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Johnston, A.M., Jenner, W N., (1976). 20.12.76. Study of the metabolism of ^{14}C-labelled N-AB 365 in the male baboon Project No. 102214 - U76-0165 -. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Kopitar, Z., (1969). 21.10.69. N-AB 365-CL Autoradiographic investigations on the distribution of [^{14}C]-N-AB 365 CL in rats and pregnant mice (ADME 1 B) - U69-0108. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Kopitar Z., (1970). 26.02.70. N-AB 365CL. Pharmacokinetic investigations with [^{14}C]-N-AB 365 CL in rats (ADME 1 B) - U69-0109 -. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Kopitar, Z., Zimmer, A., (1973). 18.12.73. Effect of repeated doses of NAB 365 CL on the pharmacokinetic profile in rats (ADME 1 D) - U73-0158 -. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

LLoyd-Evans, L.P.M., (1994). Application for establishment of maximum residue limits under council regulation (EEC) 2377/90. Clenbuterol hydrochloride; expert report on the residues file - U-Venti 138, U-Planipart 80, U-Venti TMP/S 111-. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Meyer, H.H., Rinke, L.M., (1991). The pbarmacokinetics and residues of clenbuterol in veal calves. J Anim Sci 69, 4538-4544

Rominger, K.L., Schrank, H., (1982). 28.09.82. Placental transfer of ^{14}C-clenbuterol in the dog - U82-0291. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Rose, M.D., Shearer, G., Farrington W.H.H., (1995). The effect of cooking on veterinary drug residues in food: clenbuterol. Food Addit Contam, 12, 67-76

Schmid, J., (1977). 15.12.77. Pilot pharmacokinetic investigations after intravenous administration of N-AB 365 CL in a cow - U-Venti 13, Plani 9, Venti TMP/S 18 - U77-0187 -. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Schmid, J., (1980). 13.11.80. Investigations of the placental transfer of ^{14}C N-AB 365 CL in the baboon - U80-0229. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Schmid, J., (1982). Pharmacokinetics, metabolism and tissue distribution of ^{14}C N-AB 365 CL in the baboon Report no. 2220 from Inveresk Research International - U82-0286. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Schmid, J., (1990a). 10.01.1990. Report: N-AB 365 CL. N-AB 365 CL in the cow's milk after oral and intramuscular administration (P 64189) - U-Venti 105, Plani 63, Venti TMP/S 88 - U90-0099. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Schmid, J., (1990b). 10.01.1990. Report: N-AB 365 CL. Plasma levels and residue analysis in cows and calves (P 62/89) - U-Venti 106, Plani 64, Venti TMP/S 89. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Schmid, J., Bücheler A., (1987). 07.10.87. Report: NAB 365 CL (Clenbuterol). Quantitative determination of Clenbuterol in tissues by automated gas chromatography/mass spectrometry ADME 65/87 - U-Venti 85, Plani 51, Venti TMP/S 66 - U87-0984 -. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Schmid, J., Prox, A., (1986). 24.10.86. Isolation and structural elucidation of NAB 365-CL metabolites from the dog urine - U86-0932 -. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Schmid, J., Zimmer, A., (1977a). 17.10.77. Pilot pharmacokinetic studies following oral administration (single and multiple dosing) of ^{14}C-N-AB 365 CL to a cow - U-Venti 12, Plani 7, Venti TMP/S 17 -U77-0185-. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Schmid, J., Zimmer, A., (1977b). 15.12.77. Pilot pharmacokinetic investigations after intramuscular administration (single and multiple doses) of N-AB 365 CL in the cow - U-Venti 14, Plani 8, Venti TMP/S 19 - U77-0188 -. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Schmid, J., Zimmer A., (1977c). 22.12.77. N-AB 365 CL. ADME V. Residue studies in the cow following multiple i.m. dosing with N-AB 365 CL - U-Venti 16, Plani 11, Venti TMP/S 21 - U77-0190 -. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Zimmer, A., (1971). 13.10.71. N-AB 365-CL Metabolism and species comparison in the rat, rabbit and dog (ADME IV) - U71-0091 -. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Zimmer, A., (1974a). 15.08.74. Comparison of the pharmacokinetic profile in the dog with single and repeated dosage (ADME 1 D) - U73-0161 -. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Zimmer, A., (1974b). 10.12.74. Pharmacokinetics and metabolite pattern in the rabbit and dog (ADME II) - U74-0116 -. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Zimmer, A., (1974c). 13.12.74. Metabolism in Man; comparison with dog and rabbit, using radioactive substance (ADME IV) - U74-0117 -. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Zimmer, A., (1976). 20.04.76. Administration of Clenbuterol in man. Single doses, multiple doses, and metabolite samples (ADME V) - U76-0159 -. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

Zimmer, A., (1977). 20.12.77. N-AB 365 CI ADME II, V. Preliminary investigations of pharmacokinetics in a horse - U-Venti 15, Plani 10, Venti TMP/S 20 - U77-0186. Submitted to FAO by Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany.

CYPERMETHRIN

First draft prepared by
Dr. Raymond J. Heitzman
Compton, Newbury
Berkshire, United Kingdom

IDENTITY

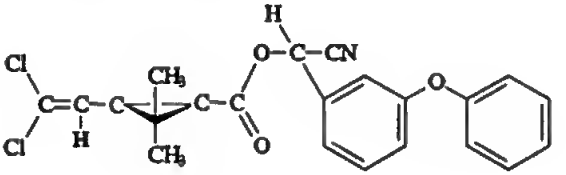
Chemical name: (RS)-alpha-cyano-3-phenoxybenzyl-(1RS,3RS,1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (IUPAC name)

(RS)-cyano(3-phenoxyphenyl)methyl(1RS)-cis-trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate (Chemical Abstracts name)

C.A.S. number: 52315-07-8

Cypermethrin is a mixture of all eight possible chiral isomers (see alphacypermethrin monograph)

Structural formula:



Molecular formula: $C_{22}H_{19}Cl_2NO_3$

Molecular weight: 416.3

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Appearance: Yellow-brown viscous liquid to semi-solid crystalline mass

Purity: The commercial preparation contains 94.2% Cypermethrin

Melting point: 80.5°C

Vapour pressure: 1.9×10^{-7} pascals at 20°C

Solubility (g/l at 20°C):

Water	9.0×10^{-6}
Cyclohexane	>600
Xylene	>600
Ethanol	>337
Hexane	103
Acetone	>450

Density:	1.23 kg/l at 20°C	
Octanol-water partition coefficient (P):	2.0×10^6	
Stability:	Hydrolytic:	Stable under acid or neutral conditions but not alkaline conditions
	Photolytic:	Stable
	Thermal:	Stable to 220°C
	Oxidation:	Stable in air at ambient temperatures

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Cypermethrin is a synthetic pyrethroid used for the control of ectoparasites which infest cattle, sheep, poultry and some companion animals. There are in progress investigations into the use of the compound to control sea-lice infestations in farmed fish. However Cypermethrin is toxic to aquatic life and it is important to avoid contamination of surface waters. Cypermethrin may be applied orally or topically (ear tag, dipping, spraying, pour-on).

Dosage

The commercial formulations are in the form of ear tags, sprays, dips and pour-on formulations.

METABOLISM

Radiolabel and metabolite nomenclature

Studies on the metabolism of cypermethrin in animals have been conducted using ^{14}C-cypermethrin labeled primarily in the rings of both the acid and alcohol portions in the molecule. These will be referred to as ^{14}C-cyclopropyl and ^{14}C-benzyl (or ^{14}C-phenoxy), respectively. The following abbreviations are used throughout: 3PBA = 3-phenoxybenzoic acid; 4HO3PBA = 3-(4-hydroxyphenoxy)benzoic acid; DCVA = 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (present as the cis- and trans-isomers); cis- and trans-HO-DCVA = DCVA hydroxylated at the cis- and trans-methyl groups, respectively.

Pharmacokinetics - Excretion

Rat

When the cis and trans isomers of cypermethrin were dosed orally to rats, both were metabolised and eliminated rapidly. For example, 98-101% of the radioactivity derived from the ^{14}C-benzyl-labeled compounds could be recovered in 3 days (Crawford and Hutson, 1977a). 53% (in males) and 66% (in females) of the dose was excreted in the urine and 27-29% in the faeces (Crawford, 1977). Tissue residues were low apart from those in the fat derived from the cis-isomer. This residue (about 1 mg/kg at a dose of 2 mg/kg) did not seem to be eliminated during the 8 dsy period of the experiment. In a follow up study the slow elimination of radioactivity in the later stages from liver and kidney was a consequence of the slow elimination from fat (Crawford and Hutson, 1978). The lipophilic character of the cis and trans-isomers are likely to be very similar and therefore it is not immediately obvious why the trans-isomer is released from fat more quickly than the cis-isomer (Crawford and Hutson, 1977a). It is possible that fat tissue contains an esterase (or lipase) that exhibits carboxylesterase activity towards the pyrethroids; trans-isomers generally are hydrolysed approximately 50 times faster than cis-isomers (Casida et al, 1976) thus the rate-limiting step in the release of cypermethrin from fat may be its metabolism in that tissue.

Cattle

Cypermethrin is used as a pesticide on crops (e.g. cotton) and the by-products may be fed to cattle. In three studies, lactating cows were fed daily for three weeks diet containing 0.2 mg of ^{14}C-Cypermethrin per kg diet (Hutson & Stoydin, 1979), or daily for one week 5 mg of ^{14}C-Cypermethrin (labels for both rings) per kg diet (Crawford, 1978) or daily for one week 10 mg of ^{14}C-Cypermethrin (label in benzyl ring) per kg diet (Hutson, 1980). Milk, faeces and urine were collected and the excretion of radioactivity monitored. An equilibrium between ingestion and excretion was reached after 3-4 days. The major routes of excretion were via the urine (49-54%) and faeces (43-44%) with <1% in the milk. Almost all (>97%) of the administered radioactivity was recovered.

Sheep

Two male sheep were topically treated (21.9 mg/kg BW) while a third was orally dosed (3.9 mg/kg BW) with labeled in the cyclopropyl and benzyl positions, see Table 3 (Crawford and Hutson, 1977b). Cypermethrin was slowly absorbed and eliminated when applied topically to sheep. Less than 0.5% of the dose was excreted in urine within 24 h and only 2% over a six day period. Faecal elimination was also slow, 0.5% of the dose being eliminated in six days. Approximately 30% of the applied dose was recovered from the application areas of both sheep. The elimination of radioactivity from the sheep, orally treated, was rapid, 61% of the administered dose being eliminated 48 b after dosing. Urinary elimination comprised 41% of the dose and faecal elimination 20.5%.

Poultry/Hens

A study in laying bens was conducted at a single dose level equivalent to 10 mg/kg in the diet, 0.7 mg/kg BW, administered twice a day for 14 consecutive days (Hutson & Stoydin, 1987). Eggs and excreta (combined urine and faeces) were collected once a day. The total output of radioactivity in the faeces (and urine) averaged 95% of the dose. The total radioactivity in whole eggs reached maximum values, 50-70 μg/kg expressed as parent drug equivalents, between 5-8 days on treatment and remained at this level for the remainder of the dosing period (see also radiodepletion studies).

Metabolism

Cattle

Methods

In general samples were extracted with organic solvents and the radioactivity measured following radio-TLC or radio-HPLC. A cream/wbey separation was carried out on milk. The content of cypermethrin in milk extracts was also measured by GC.

Urine and faecal metabolites

Three studies (see excretion section above) were carried out on lactating cows in which ^{14}C-Cypermethrin was fed in the diet. The lowest dose (0.2 mg/kg feed) did not permit a quantitatively accurate metabolite analysis; however, qualitative analysis of the urinary metabolites showed the presence of 3PBA-glutamic acid and 4HO3PBA in a ratio of 4:1. At the 5.0 mg/kg feed dose, the urinary metabolites identified were 3PBA-glutamate (68%) (confirmed by MS), 3PBA-glycine (16%), 3PBA (9%), and 4HO3PBA-sulphate (1%). Urinary metabolites at the 10.0 mg/kg dose were not analyzed. Faecal radioactivity was 88% extractable into organic solvent. TLC analysis showed that 85% of this was unchanged cypermethrin.

Tissue metabolites

Metabolite analysis on tissues was derived from the 10.0 mg/kg diet dose study (Hutson, 1980). The residue in milk was 90% extractable and shown to be cypermethrin. Both *cis* and *trans* isomers were present. Radioactive residue in fat that was removed by solvent extraction was 98%; in addition, 90% of the fat residue

was shown to be the parent Cypermethrin. The isomeric ratio was found to be 1 : 1 cis/trans in both animal matrices. The majority (80%) of the radioactive residue in muscle was extracted by methanol, but the extract was not further investigated since the residue level amounted to < 10 μg/kg. 80% radioactivity was extracted with hot methanol from liver. Hot methanol extraction was more effective compared to extraction at room temperature. The extract was further hydrolysed to liberate free metabolites, especially 3PBA and 4HO3PBA from their conjugates. The unextracted (bound) liver residue was hydrolysed in 5M HCl for about 4 hours to yield an additional 17% of the label. A level of 92% of the kidney residue could be extracted with methanol at room temperature. Bound residue in kidney was < 10 μg/kg and was not further investigated. Metabolite data derived from analysis of liver and kidney is summarized in Table 1.

Table 1. Metabolites as mg/kg of ^{14}C-Cypermethrin in bovine liver and kidney

Metabolite	Liver Extract	Liver Extract (hydrolysed)	Liver Bound Residue (hydrolysed)	Kidney Extract
Polar	0.036	0.008	0.003	0.023
3PBA-Glutamate	0.067	0.011	0.015	0.070
3PBA-Glycine	0.003	-	-	0.004
Unknown	-	0.003	0.001	0.002
4HO3PBA	0.007	0.025	0.009	0.005
Unknown	0.007	-	0.002	0.004
3PBA	0.010	0.078	0.014	0.009
Cypermethrin	0.010	-	-	0.001
Unknown	-	0.014	-	-
Aqueous phase				0.012
Unextracted				0.011
Total	**0.140**	**0.139**	**0.044**	**0.141**

Original concentration of radioactivity was 0.216 mg ^{14}C-Cypermethrin/kg, thus about 85% of the residues were isolated and approximately 77% of the residue identified. There was very little parent Cypermethrin present in the residues in liver and kidney. A significant proportion (>90%) of the bound residues could be liberated to yield the same metabolites as in the free fraction (Croucher, Hutson, and Stoydin, 1985).

Sheep

Two male sheep were topically treated (21.9 mg/kg BW) while a third was orally dosed (3.9 mg/kg BW) with labeled in the cyclopropyl and benzyl positions (Crawford and Hutson, 1977b).
The metabolite profile was not determined in sheep. However the portion of the total radioactive residue attributable to cypermethrin was measured and varied in each tissue. A higher contribution was observed in fat as compared to the muscle and organ tissues; the results are summarized in Table 2.

Table 2. Percent of total radioactive residues in sheep tissues attributable to cypermethrin

Tissue/Route of application Withdrawal period	Topical 24 h	Topical 6 d	Oral 2 d
Liver	13	17	8
Kidney	<3	<4	<1
Muscle (shoulder)	nq	nq	33
Fat (renal)	88	80	63
Fat (subcutaneous)	-	92	67

nq = not quantifiable

Hens/Poultry

About 60% of the residue in fat was present as cypermethrin in the original cis/trans isomer ratio. In contrast, the egg yolk residue consisted of 50 μg cypermethrin per kg and 100 μg other lipophilic compounds per kg. This component behaved as a lipid in that it was retained in hexane when cypermethrin was extracted from hexane into acetonitrile. It could not be separated from the natural yolk lipids during several chromatographic separations. It is relevant, in this context, that 3-phenoxybenzoic acid, when dosed to rats, appeared (in low concentration) in the skin as a mixture of triglycerides including the 3-phenoxybenzoyl dipalmitoyl glycerols (Crayford & Hutson, 1980).

Of the tissues analysed, the liver contained the highest residue (370 μg/kg) of which only 50 μg/kg was accounted for as cypermethrin. The common amino acid conjugates of 3-PBA were not substantial residual metabolites. The major excreted metabolite of 3-PBA in laying chickens, α-N-acetyl-δ-5-N-(3-phenoxybenzoyl)ornithine (Huckle et al., 1982) might be expected as a metabolite in liver, but this could not be confirmed. The hepatic metabolites appear to be very polar compounds, which cannot be converted into substantial quantities of 3-phenoxybenzoic acid or to its 4'-hydroxy derivative. Residues in breast and leg muscle (10-20 μg/kg) were too low for characterisation.

Laboratory animals

The majority of the total residues in the fat of rats after oral administration for 8 and 25 days was unchanged Cypermethrin (Crawford and Hutson, 1978). In another study rats were administered 1-2 mg/kg BW of either cis or trans ^{14}C-Cypermethrin and the metsbolites investigated. The major metabolites were in the cis form from the cis isomer, namely DCVA (36% of radioactivity) as the free or glucuronide conjugate or the parent compound (30%). Similarly for the trans isomer, 59% was trans-DCVA and parent compound (30%) (Crawford et al, 1981).

Summary

In all species there was hydrolysis of the ester bond and residues of each half of the molecule were found in different proportions in rats, cattle, poultry and humans.

In cattle the products were mostly identified as containing the phenoxy ring structure, whereas in humans and rats the cyclopropanyl derivatives were mainly identified.

Cypermethrin, is the single most significant intact pyrethroid occurring in milk, eggs, and tissues following oral administration to food producing animals.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Radiodepletion studies were carried out using equal mixtures of the cis and trans forms of ^{14}C-Cypermethrin labeled in ooe or both rings. The compound was administered orally to cattle, sheep and hens and also topically to sheep as listed in Table 3. Oral administration of Cypermethrin is not the normal method of application of the compound to farm animals and only the one study in sheep was carried out using a topical route of administration. Thus there is limited information on the residues at the sites of application, in particular there is a lack of information on residues in the subcutaneous fat layers of the skin when spray, dip and pour-on preparations were used.

Table 3. ^{14}C-Cypermethrin radiodepletion studies in cattle, sheep and poultry

Animals	n	^{14}C-Label	Route	Dose (mg/kg)	Days dosed	Sampling times	Tissues	Ref
Cows	2	B	oral	0.2 (diet)	20/21	<4 h daily	M,L,K,F,Mk	1
Cows	1	B	oral	5 (diet)	7	<4 h daily	M,L,K,F,Mk	2
	2	C	oral	5 (diet)	7	<4 h daily	M,L,K,F,Mk	
Cows	1	B	oral	10 (diet)	7	16 h daily	M,L,K,F,Mk	3
Sheep	1	BC	oral	3.9 (BW)	1	2d	M,L,K,F	4
	2	BC	topical	21.9 (BW)	1	1d, 6d	M,L,K,F,S	
Hens	4	B	oral	10 (diet) 0.7 (BW)	14	4.5 h daily	M,L,F Eggs	5

B and C are labels in benzyl ring or cyclopropyl ring respectively. Mk is milk sampled at each milking.
M = muscle; L = liver; K = kidney; F = fat; S = skin at application area.
References: 1. Hutson and Stoydin, (1979); 2. Crawford, (1978); 3. Hutson, (1980); 4. Crawford and Hutson, 1977b; 5. Hutson and Stoydin, (1987)

Cattle

The total residues of ^{14}C-Cypermethrin were measured in the edible tissues of dairy cows receiving labeled Cypermethrin in their feed as listed in Table 3. In the study where the cows received ^{14}C-Cypermethrin labeled in either of the rings, no differences in the total residues were observed for the respective tissues. The total residues of ^{14}C-Cypermethrin for the edible tissues for the three studies are shown in Table 4.

Table 4. Total residues (μg/kg) of ^{14}C-Cypermethrin in orally dosed cattle slaughtered on the last day of dosing

Reference Table 3	Dose (mg/kg feed)	Muscle	Liver	Kidney	Renal Fat	Subcut Fat
1	0.2	<1	4, 8	3, 4	10, 12	8, 9
2	5	<40	100 (3)	50-130	30-100	10-60
3	10	10	210	110	100	80

Milk

Milk was collected from the cows during the period of treatment and the radioactivity measured in the twice daily milkings. In each of the three studies the concentrations were lowest in the first day samples and then attained plateau levels throughout the period of dosing. The maximum concentrations in μg Cypermethrin equivalents/l were 1.2, 13.1 and 31 for the cows receiving 0.2, 5 and 10 mg ^{14}C-Cypermethrin/kg feed, respectively. No measurements were made after the last day of dosing. Residues in the whole milk ranged from 0.2-1.2 μg/kg with 60-70% of the radioactivity in the cream phase for the lowest dose and 11-31 μg/kg with ca 90% in the cream at the higbest dose. The extracted residue (>90%) was shown to be Cypermethrin.

Sheep

Two male sheep were topically treated while a third was orally dosed with ^{14}C-labeled cypermethrin (see Table 3) (Crawford and Hutson, 1977b). Liver, kidney, fat (from three different areas), and muscle (shoulder and rear leg) samples were assayed for total radioactivity content. Tissues were also extracted using bexane/acetone (2:1), and analyses were performed by liquid scintillation counting (LSC) with product analysis conducted using gas chromatography (GC) and limited to cypermethrin.

Residues in tissues were comparable except for liver and kidney where higher values were observed in the tissues from the orally-dosed animals. A summary of the radioactive residue values is provided in Table 5.

Table 5. Total residues (μg/kg) of ^{14}C-Cypermethrin in sheep

Route	Time post dosing (days)	Muscle	Liver	Kidney	Renal Fat	Subcut Fat
Topical	1	30-40	100	140	170	100000*
Topical	6	30-60	140	120	300	3300*
Oral	2	30-40	390	360	410	260

* At site of application

Laying Hens

Four hens were administered ^{14}C-Cypermethrin in the feed (see Table 3) twice daily for 14 days. Eggs were collected throughout the study and the birds were sacrificed 4.5 hours after the last dose. Total radioactivity and residues of Cypermethrin and some metabolites were measured in the eggs, (white and yolk), muscle, liver and fat. The results are given in Table 6.

Table 6. Residues (μg/kg) of ^{14}C-Cypermethrin in four laying hens and their eggs

	Muscle leg	Muscle breast	Liver	Fat	Whole Egg	White	Yolk
Total ^{14}C	16-25	9-14	320-410	60-110	50-70	7-10	130-190
% Cypermethrin	nm	nm	14%	56%	nm	nm	33%

The values for residues in eggs are for the maximum levels reached for each of the four hens. The residues in eggs reached plateau values about 6 days after the start of administration of radiolabel.

Rats

Male rats (430 g) and female rats (240 g) were given a single oral dose of 0.5 mg ^{14}C-Cypermethrin (cyclopropyl). The radioactivity in the tissues was determined at 3 days post-dosing. The mean values in μg/kg were; muscle, 10 (M), 9 (F); liver, 370 (M), 120 (F); kidney, 100 (M), 60 (F); fat, 310 (M), 720 (F). Although the dose is much lower in females the residues in female fat were more than twice those in males (Crawford, 1977).

Summary

Cypermethrin is the only option for the choice of parent drug as the marker compound from the radiodepletion studies. Except in fat, there was not a good correlation between the concentration of Cypermethrin and the total residues in the various tissues. Fat, milk and eggs are obvious choices as marker tissues.

Other Residue Depletion Studies (with unlabeled drug)

Residue information was provided for the recommended topical uses of the insecticide in cattle, sheep and poultry. The main residue measured was the parent compound, Cypermethrin, determined by the method of gas chromatography with electron capture detection (GC-ECD). In nearly all studies the concentrations were reported as not corrected for recovery although the recoveries were determined by spiking experiments. Other than giving the recovery data, most of the results are reported without any validation data for the methods; e.g. the LOQ were given but no information on how these were derived. Also the two detailed procedures supplied by the sponsors were without validation information McKee et al., (1981) Baldwin et al., (1977b). In all studies residues were lower in muscle, liver and kidney than in fat tissues or the fat in milk (butterfat).

Cattle

A summary of the studies is shown in Table 8.

Results

The results for the maximum concentrations found in the studies are in table 9. Residues were less likely to be found in muscle and liver, occasionally residues were observed in kidneys, residues were mostly associated with body and milk fat. There were virtually no residues when ear tags were used, some residues were seen in fat and milk fat with both the spray and dip treatments, the highest and most persistent residues were found with the pour-on preparations.

Table 8. **Residue studies with cattle**

	n	Dose	Sampling period (d)	Samples	LOQ µg/kg or l	Reference
Ear Tag	2 8	2 x 0.8 g 2 x 0.8 g	21 21	Mk-BF Mk-BF	4 4	1 1
Ear Tag	12	? x 0.8 g	1,3,8,15	M,L,K,F	5, 10(F)	2
Ear Tag	2	1 x ? g	77	M,L,K,F, Mk, Hair	10,2(Mk)	3
Spray	2	3[A] x 1.13 g	21[D]	M,L,K,F,Mk,	1, 10(F)	4
Spray	9 6	0.2 - 0.4 g 2 x 0.2-0.4 g	1,3,8 7[D]	M,L,K,F M,L,K,F	5,10(F)	5
Spray	9	2.25 g	0.5,3,7	M,L,K,F	10	6
Spray	5	0.5 g	1-10	Mk-BF	10	7
Dip	4	2[B] x 170 mg/l	4[D],14[D]	M,L,K,F	10	8
Dip	3 3	1 x 750 mg/l 2[C] x 750 mg/l	0,1,3,7 7[D]	M,L,K,F,Mk M,L,K,F,Mk	10,2(Mk)	9, 10
Pour-on	5 5	0.5 g 1.0 g	0 - 21 0 - 21	Mk Mk	2 (LOD?)	11
Pour-on	15	0.5 g	3,7,14	M,L,K,F	10	12

[A] is at 2 week intervals; [B] dipped again after 10.5 weeks; [C] dipped again after 1 week; [D] is time after last treatment; References: 1. Braun et al. (1984); 2. Bosio (1979a); 3. Wallace (1982), 4. Baldwin et al (1977c); 5. Bosio (1979b); 6. McKee (1981); 7. Solly (1988); 8. Baldwin (1977a); 9. Sherren (1979); 10. McKee (1980); 11. Roberts et al. (1987a); and 12. Roberts et al. (1987b).

Table 9. **Residues of Cypermethrin in bovine tissues, showing the maximum concentrations found in µg/kg or l**

Treatment	M, L, K	Fat	Whole Milk	Milk fat[A]
Ear Tags	None	None	None	9[1]
Spray	None	100[6]	9[4]	180[7]
Dip	None 20(K)[8]	130[8]	5[9]	n.m.
Pour-on	M 40[12] L <10[12] K 130[12]	610[12] (subcut)[B] 1400[12] (perit)	140[11]	n.m.

The superscript numbers refer to the study number given in Table 8; [A] milk fat represents about 5% of whole milk; [B] the subcutaneous fat was taken from under the area of application of Cypermethrin.

Ear tags - Studies 1-3

No residues were detected in all four edible tissues. In the first trial in Study 1, milk fat levels reached a mean level at day 3 of 5.1 μg/kg rising to a maximum mean level of 8.3 μg/kg at day 7 and declining thereafter. In the second trial, the only residues detected in milk fat were 4.2 μg/kg at day 3 in one of the eight cows and 9.2 and 8.9 μg/kg in two cows at day 21. In Study 3 no residues were detected in whole milk samples at any time.

Sprays - Studies 4-7

No residues of Cypermethrin were detected in muscle, liver and kidney at any sampling time. Residues were present in some of the fat and milk samples. The residues in fat were highest (mean 90 μg/kg) at 7 days post dosing. No measurements were made beyond this time point. In Study 6 residues of 3PBA were investigated, no evidence of this metabolite was found (LOD fat 50 μg/kg, other tissues 10 μg/kg). Residues persisted in the milk fat over the 10-day sampling period. The peak value (mean 110 μg/kg) in milk fat was at 4 days post dosing and had declined to a mean value of 30 μg/kg by day 10. In the study of residues in whole milk, no residues were detectable at 21 days post dosing.

Dips - Studies 8-10

Residues of Cypermethrin were either below or very close to the LOQ (10 μg/kg) for muscle, liver and kidney. Residues were present in renal, omental and subcutaneous fat. The concentration in the fat had not declined by 14 days post dosing, the last sampling time studied. However, the highest residue in fat was 180 μg/kg with most of the values < 100 μg/kg.

Pour-on - Studies 11-12

The residues in calf tissues are shown in Table 10. The concentrations in muscle and liver are low or not detectable. There were residues in the kidneys throughout the study period and much higher levels were found in both peritoneal and subcutaneous fat. The levels in fat were the highest recorded for any treatment. The residues although still present at 14 days were declining. The study was made using a dose which at 0.5 g per 125 kg calf was the same amount as that recommended for larger mature animals. Thus residues in larger animals may be lower.

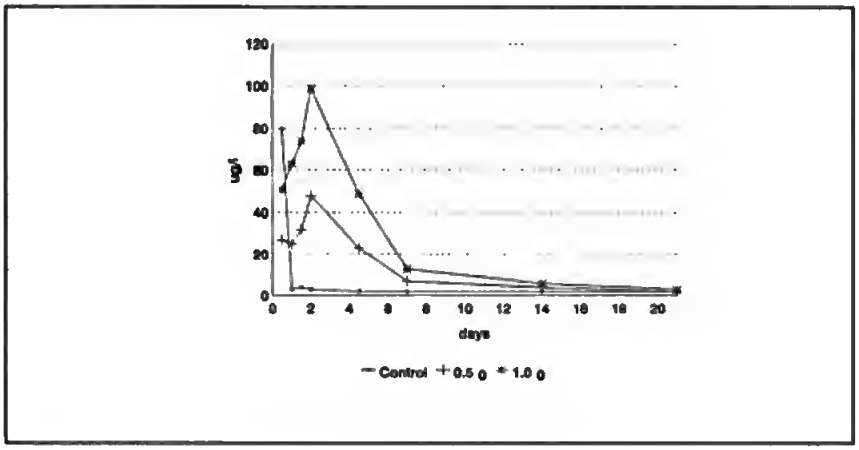
Table 10. Mean Residues of Cypermethrin in calves administered 0.5 g Cypermethrin in a pour-on preparation

Days post dosing	Muscle	Liver	Kidney	Peritoneal fat	Subcutaneous fat
3	20	< 10	50	840	470
7	< 10	< 10	70	670	260
14	< 10	< 10	40	330	140

Values in μg/kg are means of five calves per group.

Residues were measured in whole milk in Study 11 for up to 21 days post dosing. The results are plotted in Figure 1. There was an unexplained high value for the control animal at 12 hours post dosing, otherwise the residues follow a predictable pattern, with the higbest values for the higber dose and all values declining to control values within 21 days.

Figure 1. **Residues of Cypermethrin in whole milk after application of Cypermethrin in pour-on preparations**



Sheep

A summary of the studies using dips and pour-on preparations are given in Table 11.

Table 10. **Residue studies with sheep**

	n	Dose and interval (days) between dips	Sampling period[A] (days)	Samples	LOQ µg/kg or l	Reference
Dip*	12 1	0.005% 0.05%	1,3,7,14 7	M,L,K,F M,L,K,F	10 10	1 1
Dip*	3 3 5	0.01% 2 x 0.01% (4) 3 x 0.01% (4)	0 0 0,1,3,6,10	M,L,K,F M,L,K,F M,L,K,F	10 10 10	2 2 2
Dip*	12	2 x 0.005% (7)	1,2,5,7	M,L,K,F	10	3
Dip*	6	0.015%	1,3,7,10,15	Mk	5	4
Pour-on*	20 20	0.375 g 0.75 g	1,3,7,14,28 1,3,7,14,28	M,L,K,FM, L,K,F	0.2 0.2	5 5
Pour-on*	5 5	0.375 g[B] 0.375 g[C]	7 7	M,L,K,F M,L,K,F	10 10	6 6

* Results were not corrected for recovery - recoveries were normally >75%;
[A] Sampling done after last application; [B & C] Two different solvents were used;
References: 1. Baldwin (1977b); 2. McKee and Wallace (1981); 3. Wallace (1980); 4. Bosio (1981a); 5. Perret (1982); and 6. White (1987).

Residues of Cypermethrin were close to or in most cases below the LOQ in all the studies of dipped sheep. Residues were found in both the perirenal fat and omental fat. Residues were measured in the subcutaneous fat in one study only, but they were higher than in either omental or perirenal fat. Surprisingly in view of the radiometric data, residues were not measured in the subcutaneous fat at the site of application in the pour-on studies. The mean values in fats are shown in Table 12.

Table 12. Residues of Cypermethrin in fat of sheep after dipping or pour-on preparations

WT (d)	Study - see reference number in Table 11									
	1[A]			2		3	5		6	
	OF	PF	SF	OF	PF	Fat	OF	PF	OF	PF
0				70	80	50				
1	15	<10	20	110	140		20	30		
2						27				
3	10	<10	25	130	140		40	40		
5						60				
6				120	150					
7	<10	<10	<20			70	40	40	35, 18	4[R1], 10[R2]
10				60	60					
14	15	10	<20[B]				30	40		
28							20	20		

[A] Values for the recommended dose; [B] Does not include one value of 70 thought to be an outlier; [R1 & R2] Recoveries were low at 40% and 46% respectively; OF = omental fat; PF = perirenal fat; and SF = subcutaneous fat

Sheep Milk

Residues were measured in the whole milk of ewes after dipping once with a 0.015% formulation (Study reference 4, Table 11). Residues persisted throughout the 15 days post-dipping period. The mean values in μg/l at 1, 3, 7, 10 and 15 days were 13, 10, 9, 7 and 7 respectively. The milk fat was about 5.8% of the milk and if all the residues are in the fat then the maximum concentration in the fat was 206 μg/kg on day 1 and also a high value of 143 μg/kg was seen on day 7. The results were not corrected for recoveries which were 70-85%.

Poultry - Laying Hens

Cypermethrin was sprayed on domestic hens, on a single occasion, diluted with water at dosage rates of 10 and 20 mg Cypermethrin/animal. In each group animals were sacrificed at various intervals after treatment, from 1 to 14 days, and samples of tissues were taken. In addition, eggs were collected from each group, from 3-day periods, between treatment and sacrifice. All the samples were analysed for residues of cypermethrin (Bosio, 1981b). The results are given in Table 13. Residues were at or below the LOQ in muscle, liver, kidney and eggs but were present in fat and skin throughout the 14 day post dosing period. The levels in skin were

higher than those in fat.

Table 12. Residues of Cypermethrin in laying hens after spraying with Cypermethrin

	Range of Cypermethrin (μg/kg) n=3	
	Dose 10 mg	Dose 20 mg
Muscle	10 - 20	<10 - 30
Liver	<10	<10
Kidney	<10	<10 - 20
Fat	30 - 80	25 - 140
Skin	80 - 400	170 - 1300
Eggs	<10	<10

Bound Residues/ Bioavailability

There was evidence of bound residues particularly in liver and kidney, but these usually amounted to <20% in the liver and <10% in other tissues. The bound residues in the liver were treated with HCl and this liberated >90% of the radiolabel to yield metabolites similar to those in the free fraction (Croucher et al., 1985).

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES, EGGS AND MILK

Tissues

A method for the determination of Cypermethrin and 3PBA in the edible tissues was submitted. Tissue (5 g) was extracted with acetone/petroleum ether. The residuum was separated and contained 3PBA residues. After drying the extract, the dried extract was partitioned between acetonitrile and petroleum ether. The phases were separated and processed as follows:

1. 3PBA: The acetonitrile extract was made to a 40% solution in water and partitioned with petroleum ether. The aqueous phase contained more 3PBA and was combined with the first residuum. The combined phases were hydrolysed with NaOH, acidified with HCl and the 3PBA back extracted into petroleum ether. 3PBA was assayed by reverse phase HPLC.

2. Cypermethrin: The petroleum ether phase was concentrated and cleaned up by liquid solid chromatography on a Florisil column. The eluate was analysed for Cypermethrin content using GC-EC (McKee et al., (1981).

The limits of determination (LOQ) for Cypermethrin were claimed (no data) as 10 μg/kg and 50-100 μg/kg for 3PBA. Recoveries were not appended but should be measured by spiking for a batch of analyses (Baldwin et al., 1977b).

Milk

Samples of milk are treated with potassium oxalate solution, ethanol, diethyl ether and hexane. The extract is evaporated to dryness to measure the fat content of the milk. The extract is purified further by partitioning between hexane and acetonitrile, followed by column chromatography on Florisil. The residues of

Cypermethrin were determined by GC-EC. The limit of detection was claimed (no raw data) as approximately 2 μg/l milk. Recoveries of spikes of 5 and 10 μg/l were 99±8% (Baldwin et al., 1977d).

Eggs

A validated method was not supplied.

APPRAISAL

Cypermethrin is a synthetic pyrethroid insecticide applied topically to cattle, sheep and poultry. Cypermethrin is a mixture of all eight possible chiral isomers. The pharmacokinetic, metabolism and depletion studies using radiolabeled cypermethrin were carried out in cattle, sheep and poultry using oral administration (except for the topical application in two sheep) and not spray, dip or pour-on formulations.

Following oral administration of ^{14}C-Cypermethrin to cattle, poultry or rats the radioactivity was excreted rapidly in both the urine and faeces. Less than 1% of the dose was found in milk or eggs. The topical application of ^{14}C-Cypermethrin to sheep resulted in less than 3% of the dose being excreted over a six day period.

In all species after oral administration there was evidence of hydrolysis of the ester bond and residues of each part of the molecule were found in different proportions in rats, cattle, poultry and humans. There was extensive metabolism in bovine liver and kidney with the major metabolites consisting of either the conjugates or free forms of 3-phenoxybenzoic acid (3-PBA) or 4-hydroxy-3PBA. 97% of the radioactivity was extracted from liver. 92% of the residues in kidney could be extracted at room temperature. Bound residues in kidney were less than 10 μg/kg and were not further investigated. The residues in milk and fat were, respectively, 90% and 98% extractable and shown to be cypermethrin. In fat and milk the cis and trans isomers were present in an equal ration which suggests that no interconversion of the isomers occurs. No information on metabolism following absorption through the skin of cattle or poultry was provided and no residue profile data were available for sheep.

Radiolabeled depletion studies were carried out in cattle or poultry to only one day after the last oral dose and only at 1 and 6 days after topical dosing of two sheep. Thus it is not possible to determine the depletion of the total residues in these species.

The radiolabeled studies in farm animals show that the residues were higher in fat, liver and kidney (up to 410 μg/kg) than in muscle (up to 60 μg/kg). When sheep received the pour-on application the residues in the subcutaneous fat at the site of application were more than ten fold higher than following an oral dose.

Based on the limited data cypermethrin is the only residue possible for selection as a marker compound. This is suitable for fat, milk (in the milk fat) and eggs. However, the relationship between the concentration of cypermethrin and the total residues in muscle, liver and kidney was imprecise and not studied in the post-dosing period.

There were a large number of trials in which the residues of cypermethrin were measured in cattle, sheep and poultry following the recommended field uses. In all studies residues were lower in muscle, liver and kidney than in fat tissues or milk fat.

The residues following the use of ear tags impregnated with cypermethrin for cattle were mostly below the limit of quantification. The residues measured in cattle and hens after applying a spray formulation were at low levels (<10-30 μg/kg) in muscle liver, kidney and eggs. Higher residues were observed in fat (mean concentration was 90 μg/kg at 7 days post dosing) and in the milk fat (mean peak value was 110 μg/kg at 4 days post dosing) and had declined to a mean value of 30 μg/kg by day 10.

The residues of cypermethrin following the dipping of cattle in the commercial formulations were either below or very close to the LOQ (10 μg/kg) for muscle, liver and kidney. Residues were present in renal, omental and

subcutaneous fat. The concentration in the fat had not declined to the LOQ by 14 days post dosing, the last sampling time studied. However the highest residue in fat was 180 μg/kg with most of the values less than 100 μg/kg.

The residues in calf tissues were measured following the use of a 0.5 g pour-on application. The concentrations in muscle and liver are low or not detectable. There were residues (up to 130 μg/kg) in the kidoeys throughout the 14 days post dosing study period and much higher levels were found in both peritoneal and subcutaneous fat. The levels of up to 1400 μg/kg in fat were the highest recorded for any treatment. The residues, although still present at 14 days, were declining. The study was carried out using a dose of 0.5 g per 125 kg calf which was the same amount as that recommended for larger, mature animals. Thus residues in larger animals may be lower.

The residues were measured in whole milk for 21 days after applying a dose of either 0.5 g or 1 g as a pour-on preparation to lactating cows. The residues followed a predictable pattern, with the highest values (up to 168 μg/l) being reached during day 2 for the higher dose and all values declining to cootrol values (2 μg/l) within 21 days.

In sheep, the residues were measured following either the application of dip or pour-on preparations. Residues of cypermethrin were close to or in most cases below the LOQ in all the studies for muscle, liver and kidoey. Residues were found in both the perirenal fat (<10 to 150 μg/kg) and omental fat (<10 - 130 μg/kg). Residues were only measured in the subcutaneous fat (<20 - 25 μg/kg) in one study, but in this study they were higher than in either omental or perirenal fat. Surprisingly in view of the radiometric data, residues were not measured in the subcutaneous fat at the site of application in the pour-on studies. Residues were measured in the whole milk of ewes after dipping once using a 0.015% formulation. Residues persisted throughout the 15 days post-dipping period. The mean values in μg/l at 1, 3, 7, 10 and 15 days were 13, 10, 9, 7 and 7, respectively. The milk fat was about 5.8% of the milk and if all the residues are in the fat then the maximum concentration in the fat was 206 μg/kg on day 1 and a high value of 143 μg/kg was seen on day 7 also. The results were not corrected for recoveries which were 70-85%.

Residues were measured in hens over a 14 day period after applying either a dose of 10 or 20 mg per bird as a spray. Residues were at or below the LOQ in muscle, liver, kidney and eggs but were present in fat (25 - 140 μg/kg) and skin (80 - 1300 μg/kg).

There were low concentrations of bound residues and >90% of the bound material could be chemically released and shown to be metabolites.

Two detailed analytical methods were suhmitted, one for cypermethrin (LOQ was 10 μg/kg) and 3-phenoxy benzoic acid (LOQ was 50 μg/kg) in tissues and one for cypermethrin (LOD was 2 μg/l) in milk. Confirmatory methods using GC-MS are cootained in the suhmitted papers. The analytical methods were submitted without adequate validation data. Evidence is required of the LOQ and LOD of the methods.

Maximum Residue Limits

The JMPR in 1981 set an ADI for Cypermethrin of 0.05 mg/kg/day which equates to a daily intake of 3 mg for a 60 kg person.

In recommending MRLs the Committee took account of the following factors:

- The ADI is 0-50 μg/kg equivalent to 0-3000 μg for a 60 kg person. The ADI equates with that established by JMPR;

- The marker residue is parent drug, cypermethrin;

- Fat, milk and eggs are marker tissues hut muscle, liver and kidney should be considered;

- The metabolism and radiodepletion studies are not adequate and, therefore, very conservative estimates of the marker compound as a percentage of total residues in all food species is proposed. The percentages proposed for the estimation in individual tissues of total residues from the parent drug are; muscle, 30; liver, 10; kidney, 5; fat, 60; milk, 80; eggs, 30;

- There is adequate residue information from the non-radiolabelled studies using the recommended formulations; and

- There are available analytical methods, however, evidence of adequate validation are needed;

The Committee recommends temporary MRLs for cattle, sheep and poultry of 200 μg/kg in muscle, liver and kidney, 1000 μg/kg in fat, 50 μg/kg for cattle whole milk and 100 μg/kg for eggs expressed as parent drug.

Estimates of residue intake are tabulated as follows:

Tissue	Food Basket (g)	MRL (μg/kg)	μg	Percent UD/TR	Intake (μg)
Muscle	300	200	60	30	200
Liver	100	200	20	10	200
Kidney	50	200	10	5	200
Fat	50	1000	50	60	83
Milk	1500	50	75	80	94
Eggs	100	100	10	30	33
				Total	**810 μg**

UD is unchanged drug; TR is total residues

The JMPR food basket takes approximately 300 μg leaving 2700 μg. The above MRLs accommodate the ADI and the recommended use of this compound as a veterinary drug and as a pesticide.

The Committee requires the following information:

1. Radiodepletion studies which extend beyond the recommended withdrawal times and using the drug in its topical formulation. The study should determine the depletion of the total residues and the parent drug;

2. Evidence to verify the limited information of no-interconversion of isomeric forms during metabolism in the target species; and

3. Further information on the validation of the analytical methods; particulary data on the derivation of LOD and LOQs.

The committee will need to ascertain the contribution of ingested pesticide from non-food animal sources and subtract this from the ADI to calculate the allowed ADI for Cypermethrin from food animals.

REFERENCES

Baldwin, M.K., (1977a). TLGR 0115.77. Residues of the pyrethroid insecticide WL 43467 in tissues of cattle following a dip-bath application. [58]

Baldwin, M.K., (1977b). TLGR 0040.77. Residues of the pyrethroid insecticide WL 43467 in tissues of sheep following a dip-bath application. [63]

Baldwin, M.K., Buckwell, A.C. and Lad, D., (1977c). TLGR.0112.77. Residue data following the application of WL 43467 for nuisance fly control on cattle. [54]

Baldwin, M.K. et al., (1977d). SAMS 265-1 Determination of residues of the pyrethroid insecticides WL 43467 (NRDC 149) (i) or of WL43479 (NRDC 143) (ii) in milk. [71]

Bosio, P.G., (1979a). BEGR.79.116. Residues of Barricade (cypermethrin) in cattle from Australia.. [52]

Bosio, P.G., (1979b). BEGR.79.117 Residues of Barricade (Cypermethrin) in cattle from Australia. Overspray Experiments. [55]

Bosio, P.G., (1981a). BEGR81.178. Residues of Cypermethrin in sheep milk from France. 1981 Trials. [66]

Bosio, P.G., (1981b). BEGR81.131. Residues of Barricade (cypermethrin) in poultry from the UK. 1981 Trials. [69]

Braun, H.E., Frank, R. and Miller, L.A., (1984). CAND 84.001 Residues of Cypermethrin in milk from cows wearing impregnated ear tags. [51]

Casida, J. E., Ueda, K., Gaughan, L. C., Jao, L.T. and Sutherland, D.M., (1976). Structure-biodegradability relationships in pyrethroid insecticides, Arch. Environ. Contam. Tox. , 3, 491-500.

Crawford, M. J., (1977). The metabolism of WL 43467 in mammals. The fate of a single oral dose of [^{14}C-cyclopropyl]WL 43467 in the rat. Group Research Report TLGR.0004.77

Crawford, M. J., (1978). TLGR.0029.78 The Excretion and Residues of Radioactivity in Cows Treated Orally with ^{14}C-labelled WL 43467. [46]

Crawford, M. J., Croucher, A.; Hutson, D. H., (1981). Metabolism of cis- and trans-cypermethrin in rats. Balance and tissue retention study (Insecticide), J. Agric. Food Chem. 29, 130-135.

Crawford, M. J. and Hutson, D. H., (1977a). The metabolic fate of of WL 43467 (cypermethrin). Metabolism and elimination of ^{14}C-aryl-labelled cis and trans isomers in rats. TLGR 0131.77.

Crawford, M. J. and Hutson, D. H., (1977b). TLGR.0098.77. The elimination and retention of WL 43467 when administered dermally or orally to sheep. [50]

Crawford, M. J. and Hutson, D. H., (1978). TLGR.0078.78. The elimination of residues from the fat of rats following the oral administration of [^{14}C-Benzyl]WL 43481 (Cis-WL 43467) [48]

Crayford, J.V. and Hutson, D.H., (1980). Xenobiotic triglyceride formation. Xenobiotica, 10, 349-354

Croucher, A., Hutson, D.H. and Stoydin, G., (1985). Excretion and Residues of the Pyrethroid Insecticide Cypermethrin in Lactating Cows, Pestic. Sci. 1985, 16, 287-301.

Huckle, K.R., Stoydin, G., Hutson, D.H., Millburn, P., (1982). Formation of an N-acetylornithine conjugate of 3-phenoxybenzoic acid in the chicken (Pyrethroid insecticides), Drug Metab. Disposit. 10, 523-528.

Hutson, D. H., (1980). TLGR.80.121. The metabolic fate of cypermethrin in the cow. Elimination and residues derived from ^{14}C-benzyl label. [47]

Hutson, D.H. & Stoydin, G., (1979). TLGR.0075.76. The Excretion of Radioactivity from Cows Fed with Radioactively Labelled WL 43467. [45]

Hutson, D.H. & Stoydin, G., (1987). Excretion and residues of the pyrethroid insecticide Cypermethrin in laying hens, Pestic. Sci. 18, 157-168. [49]

McKee, J., (1980). BLGR. 80.021. Residues of Barricade (cypermethrin) and Supona (chlorfenvinphos) in cattle from Australia. [60]

McKee, J., (1981). SBGR.81.029. Residues of Barricade (cypermethrin) and its metabolite (3-phenoxybenzoic acid) in cattle from South Africa. [56]

McKee, J. and Wallace, B.G., (1981). SBGR81.145. Residues of Barricade (cypermethrin) and Supona (chlorfenvinphos) in sheep tissues from Australia. [64]

McKee, J., Wallace, B.G. et al., (1981). SAMS 341-1. Determination of residues of Barricade (cypermethrin) and its metabolite 3-phenoxybenzoic Acid (3-PBA) in animal tissues. [70]

Perret, G.R., (1982). ASTL.82.001. Report on residues of Cypermethrin in sheep. [67]

Roberts, N.L. et al., (1987a). SLL 97/861718. Milk residues of cypermethrin following topical administration of cypermethrin pour-on formulation SF 06646 to dairy cows. [61]

Roberts, N.L. et al., (1987b). SLL 97/861718. Tissue residues of cypermethrin following topical administration of cypermethrin pour-on formulation SF 06646 to calves. [62]

Sherren, A.J., (1979). BLGR.79.059. Residues of Barricade and Supona in Cattle from Australia Part I - Analysis of milk. [59]

Solly, S.R.B., (1988). NZL.88.001. Residues of cypermethrin in cattle milk in New Zealand. [57]

Sponsor review of metabolism, Crop Protection Division (19??)

Wallace, B.G., (1980). BLGR80.108. Residues of Supona and Barricade in Sheep from South Africa. [65]

Wallace, B.G., (1982). SBGR82.314. Cypermethrin - A residue transfer study with lactating cows and Flectron fly tags. [53]

White, D.A., (1987). NZL.87 001. Report on residues of Cypermethrin and Alphacypermethrin (proposed) in sheep tissues. [68]

ALPHACYPERMETHRIN

First draft prepared by
Dr. Raymond J. Heitzman
Compton, Newbury
Berkshire, United Kingdom

IDENTITY

Chemical names:	International Union and Pure and Applied Chemistry (IUPAC) name: A racemate comprising (S)-alpha-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate and (R)-alpha-cyano-3-phenoxybenzyl (1S,3S)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate; and a racemate comprising (S)-alpha-cyano-3-phenoxybenzyl (1R)-cis-3-(2,2-dichloro-vinyl)-2,2-dimethylcyclopropane carboxylate and (R)-alpha-cyano-3-phenoxybenzyl (1S)-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate. WL85871, alphacypermethrin (alphamethrin and alfoxylate are non-official names).
Common trade names:	FASTAC®, CONCORD®, FENDONA®, RENEGADE®.
C.A.S Number:	67375-30-80 (correct stereochemistry)
Structural formula:	See next page
Molecular formula:	$C_{22}H_{19}Cl_2NO_3$
Molecular weight:	416.3

OTHER INFORMATION ON IDENTITIES AND PROPERTIES

Appearance:	White-to-cream crystalline solid
Odour:	Mild chemical
Stability:	Highly stable to light and elevated temperatures. It is resistant to acidic hydrolysis but undergoes ester cleavage in environmental (basic) aquatic conditions. It has optimum stability at pH = 4. Its low solubility in water indicates a low bioavailability in aquatic situations.
Melting point:	81.5°C (pure material)
Melting range:	81.4-84.0°C (pure material)
Boiling point:	200°C at 9.31 PA
Vapour pressure:	3.4×10^{-7} Pascals at 25°C (pure material)
Octanol-Water partition coefficient:	$p = 3.16 \times 10^5$
Refractive index:	0.19 x C + 1.344 (C = concentration in kg/l up to 0.25, only in acetonitrile)

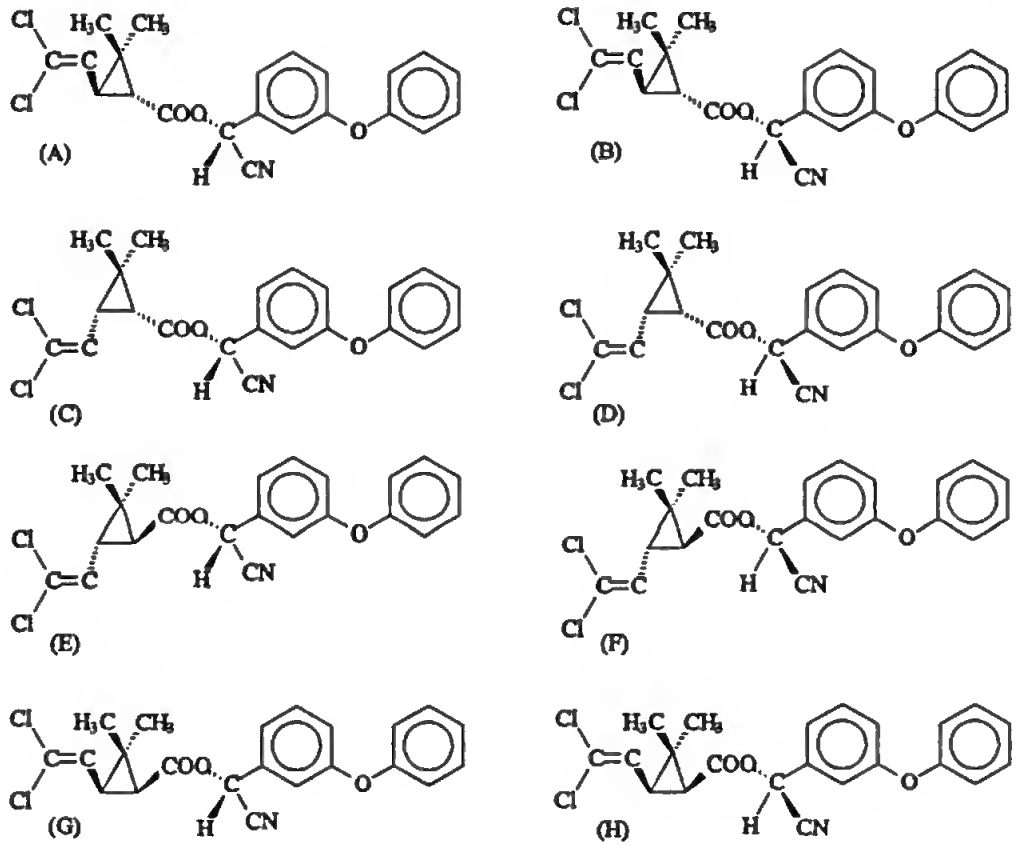
True density: 1330 kg/m³ (typical for pure material)

Solubility (g/l at 21°C):

n-Hexane	6.5
Propanol-2	9.6
Methanol	21.3
Ethyl acetate	584
Toluene	596
Fat	78
Water	2.06 μg/l at 20°C

Alphacypermethrin was determined to be miscible with acetone and dichloromethane at room temperature

Structural formula:



Chemical structure of eight cypermethrin stereoisomers. Alphacypermethrin comprises the (D) and (G) isomers.

(A) (1R,*trans*) (αR); (B) (1R,*trans*) (αS); (C) (1R,*cis*) (αR); (D) (1R,*cis*) (αS); (E) (1S,*trans*) (αR); (F) (1S,*trans*) (αS); (G) (1S,*cis*) (αR); and (H) (1S,*cis*) (αS)

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Cypermethrin is s racemic mixture of eight isomers. Alpha cypermethrin is a pyrethroid insecticide consisting essentially of two of the four cis isomers comprising cypermethrin. Alphacypermethrin is a highly active broad spectrum insecticide, effective by contact and ingestion against target pests. It is widely used in agricultural crops, forestry as well as in public and animal health.

Dosage

Applied as a pour-oo preparation for cattle and sheep, as a dip for sheep, and as a spray for poultry.

METABOLISM

Pharmacokinetics

Excretioo

Rats

The cis and trans isomers of ^{14}C-alphacypermethrin labeled in the benzyl ring were separately administered to rats by the oral route. The cis pair of isomers were in a single dose of 1.7-2.5 mg/kg body weight (BW) to 6 females and 6 males. Over an eight day collection period 50-60% of the dose was excreted in the urine and 30-40% in the faeces. The majority of the radioactivity was excreted within 48 hours followed by a persistent elimination over the remaining six days. This was different from the trans isomers where the excretion was virtually complete within 72 hours (Crawford and Hutson, 1977). The difference was due to the slower hydrolysis of the cis isomers in fat tissues.

Cattle

^{14}C-Alphacypermethrin labeled in the benzyl ring was administered orally to one lactating cow via 8 twice daily doses added to a portion of the animal's protein diet at s target dose level of 250 mg/day, ca 19 mg/kg BW. The major route of excretion of radioactivity was vis faeces, accounting for 34% of the total administered dose. A further 23% of the total administered radioactivity was excreted via urine, whereas secretion in milk accounted for < 1%. At the end of the 8 days study period approximately 58% of the total administered dose was recovered.

Poultry

No information.

Metabolism In Food Animals

Cattle

After oral dosing of ^{14}C-alphacypermethrin to one lactating cow (see above), residue concentrations (μg equiv/kg) were found in: muscle 19-29: liver, 560: kidney, 220: fat, 390-480: milk (up to 200) (Morrison and Richardson, 1994). Liver and kidney cootained a range of components. The liver extract contained at least eight metabolites with a broad range of polarities, one component (16% of the profile) had similar chromatographic properties to alphacypermethrin. The kidney extract contained at least nine metabolites with a broad range of polarities, one compooent (20% of the profile) had similar chromatographic properties to alphacypermethrin. Muscle, fat and milk contained mainly s single component (muscle 85%, fat 91% and milk 97% of the extract profile), which in each case had similar chromatographic properties to alphacypermethrin.

The ^{14}C-metabolites in urine (72-96 h after initial dosing) were characterised by co-chromatography using HPLC and TLC. The two major components (44% and 20% of the profile) had identical chromatographic properties (HPLC) to ^{14}C-3PBA glutamic acid conjugate and ^{14}C-3PBA glycine conjugate, respectively. A minor component (3% of the profile) had identical chromatographic properties (HPLC) to ^{14}C-3PBA (3-phenoxybenzoic acid).

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Two radiolabeled depletion studies in cattle (Redgrave et al., 1992; Cameron et al., 1993) were carried out, one with oral dosing and one using the pour-on application at the recommended dose (morrison and Richardson, 1994). The studies are outlined in Table 1. The results for the pour-on veterinary drug preparation are more applicable for the evaluations by JECFA.

Table 1. Radiolabeled depletion studies in dairy cattle using ^{14}C-Alphacypermethrin pour-on applications

Dose	Route	No. cows	Tissues	Sampling time (days post dose)	Reference
0.125 g b.i.d 4 d	oral	1	M, L, K, F, Milk	0.25 (Tissues)	1
0.150 g	pour-on	4	M, L, K, F	7, 14, 28, 35	2
0.150 g	pour-on	4	Milk	0 - 35	2

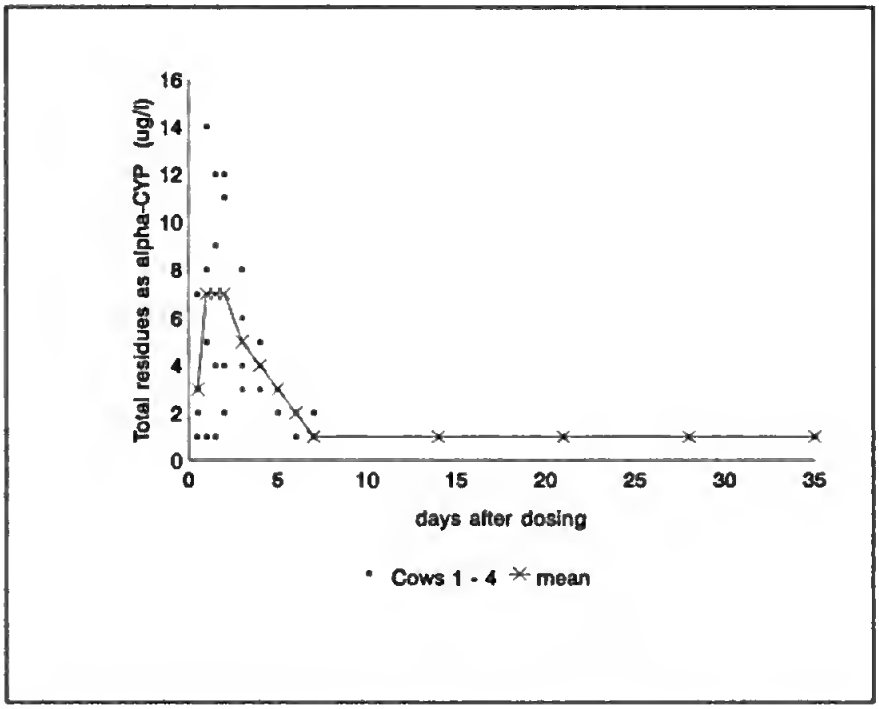
1. Morrison and Richardson, 1994; 2. Redgrave et al., 1992; b.i.d. = twice daily.

Milk

The concentrations of radioactivity detected in whole milk after oral dosing collected in the afternoon (34-199 μg equivalents/kg) were higher than in the larger morning samples (14-85 μg equivalents/kg) due to the difference in the sample size. Highest levels of radioactivity in fractions of whole milk were detected in cream (1100 μg equivalents/kg) and represented 93% of the radioactivity in whole milk.

The milk samples following the pour-on application were analysed either for total radioactivity or by GC for alphacypermethrin itself [Note: The information on the measurement of cold alphacypermethrin is not given in the reference but only in the expert report]. The lower limits of determination for the two methods were 1 μg/l and 2 μg/l for radio counting and GC respectively. Mean levels rose to 7 μg/l as determined by radio counting and to 5 μg/l as determined by GC, by day 2. Ranges for the individual animals by the two methods, respectively, were $<$1-12 μg/l and $<$2-9 μg/l. Levels determined by radio counting fell to just on the limit of determination of 1 μg/l by day 7 (Redgrave et al., 1992). The individual and the mean values for the total residues are shown in Figure 1.

Figure 1. Total residues of radiolabeled alphacypermethrin in milk after a pour-on application



Edible Tissues

At 6 h following the final oral dose administration, the highest levels of radioactivity were found in liver, renal fat, omental fat, subcutaneous fat and kidney (560, 480, 430, 390 and 220 μg equiv/kg, respectively). Compared to plasma (80 μg equiv/kg), these levels were significantly higher. All muscle samples contained levels of radioactivity < 30 μg. High levels of total radioactivity were observed in bile (5212 μg equiv/l).

The total residues (measured as radioactivity) in the edible tissues after the pour-on application were mostly below the LOQ (Redgrave et al., 1992). The LOQ varied between 10 and 30 μg/kg. Only in the subcutaneous fat of one cow slaughtered at 35 days post dosing was a residue measured at the LOQ of 30 μg/kg. Renal and subcutaneous fat samples from cows sacrificed at 7 and 14 days after treatment were also analysed for alphacypermethrin by GC with a LOQ of 10 μg/kg but only trace amounts were found. Levels in cow 1 were < 10 and 10 in renal and subcutaneous fat, respectively. Corresponding figures for cow 2 were both 20 μg/kg. These figures are not significantly different from the radio counting estimates. Thus the highest level observed in this study was about 30 μg/kg body fat.

Other Residue Depletion Studies (with unlabeled drug)

The studies carried out by the sponsor using the recommended preparations in cattle, sheep and poultry are summarised in Table 2. Residues of alphacypermethrin were measured by GC-ECD with LOQs of 10-30 μg/kg in tissues and 1-2 μg/kg in milk. The results were not corrected for recovery although the recoveries were determined in each study.

Table 2. **Residue studies using unlabeled alphacypermethrin**

Species	Route	No	Dose	Samples	Time of sampling	Reference
Cow	pour-on	5 5 5	0.1 g 0.15 g 0.2 g	Milk Milk Milk	1,2,3,4,7,14,21 1,2,3,4,7,14,21 1,2,3,4,7,14,21	1
Calves	pour-on	15	0.16 g	M,L,K,F	3,7,14	2
Calves	pour-oo	11[A] 11[a]	0.15 g 0.15 g	F (sc & perirenal)	3,7,14,21,28	3
Sheep	pour-on	3	0.2 g	F (sc), fleece, skin	3,7,14	4
Sheep	dip	3	60 mg/l dip	F (sc), fleece, skin	3,7,14	4
Sheep	pour-on	5 5	0.01 g/kg BW 0.02 g/kg BW	F (omental, perirenal)	7 7	5
Poultry	spray	40	?	Egg	2,5,10,14	6

[A] calves were aged 4-5 mooths and weighed 129-164 kg; [a] calves were aged 8-9 months and weighed 242-271 kg; sc = subcutaneous; References: 1. Sherren, A.J. (1988a); 2. Sherren, A.J. (1988b); 3. Cameron et al., (1993); 4. Francis and Gill, (1989); 5. White, D.A. (1987); 6. Sogeval, (1992).

Calves, aged 4-5 months, were dosed with 0.15 g of the pour-on preparation of alphacypermethrin and residues were determined in tissue samples by GC-ECD (LOQ 10 μg/kg) (Redgrave et al., 1992). The results are shown in Table 3.

Table 3. **Residues (μg/kg) of alphacypermethrin in edible tissues of calves after application of a pour-on preparation - dose 0.15 g**

	+3 days	+7 days	+14 days
Muscle	<LOQ	<LOQ	<LOQ
Liver	<LOQ	<LOQ	<LOQ
Kidney	<10 - 20 [10]	<10 - 30 [20]	<10 - 10 [10]
Subcutaneous fat	30 - 140 [70]	20 - 130 [80]	10 - 20 [10]
Perirenal fat	160 - 340 [250]	220 - 310 [270]	60 - 150 [90]

The values are the ranges for five calves with the means in brackets.

The depletion of residues in both subcutaneous and perirenal fat was followed in two groups of female calves treated with pour-on applications of 0.15 g alphacypermethrin (see Table 2). The results are shown in Table 4.

Table 4. **Residues of Alphacypermethrin (μg/kg) in calves and heifers after the application of 0.15 g in a pour-on preparation**

Days post dosing	Young calves age 4-5 months		Heifers aged 8-9 months	
	perirenal fat	subcutaneous fat	perirenal fat	subcutaneous fat
3	140	20	100	80
7	90	20	80	50
14	70-90	10-20	20-100	20-80
21	60-80	<10-20	40-60	10-50
28	<10-20	<10-20	10-40	10-30

Milk

The residues in bovine milk were measured on a total daily milk sample collected from cows treated with 3 different doses of a pour-on preparation (Sherren, 1988a). The results are shown in Table 5. The maximum residues were observed between days 2 and 5 after treatment and were all <LOQ by day 21. The maximum value was 5 μg/l on the 2nd and 4th day after treating with the highest dose. The profile for the residues after treatment with the recommended dose of 0.15 g follows closely that seen with the radiolabeled study (Redgrave et al., 1992).

Table 5. **Residues of alphacypermethrin (μg/l) in whole bovine milk after application of three doses of a pour-on preparation**

Dose/day	+1 d	+2 d	+3 d	+4 d	+7 d	+14 d	+21 d
0.1 g	<2 (5)	4, <2(4)	2 <2(4)	<2 (5)	2 <2(4)	2 <2(4)	<2 (5)
0.15 g	2 <2(4)	3 <2(4)	3 , 4 <2(3)	2, 2, 2 <2(2)	2 <2(4)	2 , 3 <2(3)	<2 (5)
0.2 g	3 <2(4)	2, 2, 2, 2, 5	2, 2, 3, 3, 3	2, 3, 3 3, 5	2, 2, 2, 3, 3	<2 (5)	<2 (5)

The numbers in parentheses are the number of animals.

Sheep

Sheep were treated with either a pour-on application or a dip formulation of alphacypermethrin (see study reference 4 in Table 2) and the residues in fat, skin and wool, measured in single sheep at 3, 7 and 14 days post-treatment. The results for fat and skin are shown in Table 6.

Table 6. Residues of alphacypermethrin (μg/kg) in sheep after treatment with either a pour-on application or a dip formulation of alphacypermethrin

Time after dosing (days)	Fat (sc) Pour-on	Fat (sc) Dip	Skin Pour-on	Skin Dip
3	20	20	nm	nm
7	< 10	40	20	1400
14	< 10	40	150	300

The values are not corrected for recovery. Recoveries in fat were 96 and 106 % and in skin 78 and 80 %.

In a further study (White, 1987) sheep were treated with a pour-on application at either 0.01 g/kg BW or twice this dose (see Table 2). Residues were determined in the omental and perirenal fat at 7 days after dosing. The results are shown in Table 7.

Table 7. Residues of alphacypermethrin (μg/kg) in sheep fat 7 days after treatment with pour-on applications of alphacypermethrin

Dose (g/kg bw)	Omental Fat	Perirenal Fat
0.01	3 - 11 [6]	<0.2 - 8 [3]
0.02	2 - 19 [6]	5 - 18 [10]

The values are not corrected for recovery. Recoveries in omental fat were 69 and 88 % and in perirenal fat 73 and 86 %. The values are the ranges with the mean values in brackets.

Poultry

A study was carried out of the residues in eggs after a spray application of alphacypermethrin to hens (see Table 2 and Sogeval, 1992). Only the results are submitted. The residues were measured in the albumen, yolk and whole egg and also in the yolks collected from five hens sacrificed at 14 days after dosing. The results for the eggs are shown in Table 8. The residues in the yolks at sacrifice were <5, <5, 23, 19 and 25 μg/kg.

Table 8. Residues of Alphacypermethrin (μg/kg) in eggs and yolks after application of a spray of Alphacypermethrin

Time after dosing (days)	Albumen	Yolk	Whole egg
2	<5 [<5]	<5 - 45 [12]	<5 - 13 [4]
5	<5 [<5]	6 - 43 [26]	8 - 15* [11]
10	<5 [<5]	<5 - 43 [19]	<5 - 13 [7]
14	<5 [<5]	5 - 47 [16]	<5 - 10 [7]

The values are probably not corrected for recovery. The values are the ranges with the mean values in brackets. The authors have used the LOD at 5 μg/kg and not the LOQ of 20 μg/kg for quantification! * The range excludes one value of 24 which was claimed to be an outlier.

Bound Residues/Bioavailability

In the studies on Cypermethrin (see the monograph) there was evidence of bound residues particularly in liver and kidney, hut these usually amounted to <20% in the liver and <10% in other tissues. The bound residues in the liver were treated with HCl and this liberated >90% of the radiolabel to yield metabolites similar to those in the free fraction (Croucher et al., 1985). Thus by analogy it was probable that bound residues of alphacypermethrin were quantitatively similar.

Marker Substance and Target Tissue

The only possihle option for a marker substance is the parent compound, alphacypermethrin. It represents the majority of the residue in muscle, milk and fat. Two tentative values of 16% in liver and 20% in kidney were determined in one cow treated orally. Thus there is really no indication of the ratio of alphacypermethrin to total residues in these tissues, nor in skin. However the total residues, using topical application, were below the LOQ in liver and kidney and therefore the sensitivity of tbe analytical methods could be considered in setting an MRL for these tissues. For edihle tissues fat is the first choice for a target tissue, the type of fat may not be too important. Obviously milk or milk fat and the yolk of eggs are suitahle for monitoring milk and eggs.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES, EGGS AND MILK

Milk

A gas liquid chromatographic (GC) method was used to determine alphacypermethrin residues in milk (SAMS 456-1). Samples of milk were treated with potassium oxalate solution and ethanol and extracted with diethyl ether and hexane. The extract was evaporated to dryness, the residue dissolved in hexane and the solution was passed through an Extrelut extraction column. Further clean up was obtained by using a Cyano Bond Elut cartridge. The isomers were separated and the residues determined hy GC with electron capture detection (GC-ECD). Confirmation was hy combined gas chromatography mass spectrometry (GC-MS) monitoring the ions 207 and 209 in the negative ion chemical ionization mode. The recovery of the method over a range of 5 to 20 μg/l alphacypermethrin was 95 to 105% and the limit of determination was claimed as 1 μg/l although no validation data other than for recoveries were provided.

Tissues

Method SAMS 461-1 is a GC method to determine alphacypermethrin residues in animal tissues (liver, kidney, muscle and fat). Samples were extracted hy boiling with a mixture of acetone and bexane. The solvent was evaporated and the residue redissolved in hexane. For fat and muscle, a portion of the extract was partitioned with acetonitrile hy using an Extrelut extraction cartridge. A normal hexane/acetonitrile partition was used for all liver and kidney extracts. Extracts were further cleaned up by liquid-solid chromatography on a Florisil cartridge before analysis with GC-ECD. Residues were confirmed by GC-MS. The recovery in fat is 80 to 115%, in muscle 85 to 100%, kidney 85 to 95% and liver 80 to 95%. The limit of detection is 10 μg/kg although no validation data other than for recoveries were provided (SAMS 461-1).

All of the results for the cold residues were not corrected for recoveries. Recoveries were given in the references and were mostly >85%. Thus all the values quoted are about 10-20% on the low side of the actual corrected content.

APPRAISAL

Alphacypermethrin is a pyrethroid insecticide consisting essentially of two of the four cis isomers comprising cypermethrin. It is applied as a pour-on preparation for cattle and sheep, also as a dip for sheep and as a spray for poultry.

In rats 50-60% of a radiolabeled dose was excreted in the urine and 30-40% in the faeces over an eight-day

collection period. ^{14}C-Alphacypermethrin was administered orally to one lactating cow. The major route of excretion of radioactivity was via faeces, accounting for 34 % of the total administered dose. A further 23 % of the total administered radioactivity was excreted via urine, whereas secretion in milk accounted for < 1 %.

The residues in liver and kidney contained a range of components. The liver extract contained at least eight metabolites with a broad range of polarities, one component (16 % of the residues) had similar chromatographic properties to alphacypermethrin. The kidney extract contained at least nine metabolites with a broad range of polarities, one component (20 % of the residues) had similar chromatographic properties to alphacypermethrin. Muscle, fat and milk contained mainly a single component (muscle 85 %, fat 91 % and milk 97 % of the extracted residues), which in each case had similar chromatographic properties to alphacypermethrin. The metabolite profiles were not determined in sheep or poultry.

A radiolabeled depletion study was carried out using the pour-on application at the recommended dose administered to four cows. Residues were measured at 7, 14, 28 and 35 days after dosing. The total residues (measured as radioactivity) in the edible tissues were mostly below the LOQ (10-30 μg/kg). Only in the subcutaneous fat of one cow slaughtered at 35 days post dosing was a residue measured at the LOQ of 30 μg/kg. Perirenal and subcutaneous fat samples from the cows sacrificed at 7 and 14 days after treatment were also analysed for alphacypermethrin by GC with a LOQ of 10 μg/kg but, only trace amounts were found. Levels at 7 days were < 10 and 10 μg/kg in renal and subcutaneous fat, respectively, and corresponding figures for the cow sacrificed at 14 days were both 20 μg/kg. These figures are not significantly different from the radio counting estimates. Thus, the highest level of residues observed in this study was about 30 μg/kg body fat. In the milk samples following the pour-on application, total radioactivity mean levels rose to 7 μg/l by day 2 with the range < 1-12 μg/l for the individual animals. Levels fell to just on the limit of determination of 1 μg/l by day 7.

Residues as alphacypermethrin were measured in cattle, sheep and poultry after the topical application of unlabeled alphacypermethrin. The results were submitted uncorrected for recovery. After the application of the pour-on preparation no residues were detectable in the muscle and liver of young cattle and were <30 μg/kg in kidney over the 14 day post dosing period. In two of the studies there was evidence of persistence of residues in both subcutaneous fat and perirenal fat of calves. The residues were highest (range 10-140 μg) during the first two weeks after treatment but then declined to about 10 to 40 μg/kg by day 28.

Residues in bovine milk after the pour-on treatment reached peak values between days 2 and 5 after dosing and were all below the LOQ by day 21.

Sheep which were dipped in a preparation of alphacypermethrin had higher residues in the fat, wool and skin than those receiving the pour-on application. In one study with the pour-on treatment, residues in fat were not detectable within 7 days of dosing but in sheep which were dipped the residues were 40 μg/kg in fat at 7 and 14 days after dosing. High residues were found for at least two weeks in the skin after both treatments. In another study residues at 7 days after a pour-on application were present in both perirenal fat (max. 18 μg/kg) and omental fat (max. 19 μg/kg).

In a poultry study, hens were treated with an alphacypermethrin spray and residues in eggs measured over a 14 day period. No alphacypermethrin was found in the albumen but residues persisted in the yolk for the 14 day study period.

Suitable methods for the specific analysis of alphacypermethrin in milk and the edible tissues were submitted. The methods were GC with electron capture detection followed by confirmation with GC-MS. The claimed LOQ were 1 μg/l for milk and 10 μg/kg for tissues. Recoveries were between 80 and 105 %.

Based on the available data, the only possible option for a marker substance is the parent compound, alphacypermethrin. It represents the majority of the residue in muscle, milk and fat. Two tentative values of 16 % in liver and 20 % in kidney were determined as alphacypermethrin as a percentage of total residues in one cow treated orally. Thus there is limited information on the ratio of alphacypermethrin to total residues in these tissues or in skin. However, using topical application the total residues were below the LOQ in liver and kidney and, therefore, the sensitivity of the analytical methods could be considered in recommending an MRL for these

tissues. For edible tissues fat is the first choice for a marker tissue. Whole milk or milk fat are suitable for monitoring milk and the yolk of eggs for monitoring eggs.

Maximum Residue Limits

In recommending MRLs the Committee took account of the following factors:

- The ADI is 0-20 μg/kg, equivalent to 0-1200 μg per 60 kg person;

- The marker residue is parent drug, alphacypermethrin;

- Fat, milk and eggs are target tissues but muscle, liver and kidney should be considered;

- The metabolism of the two isomers forming alphacypermethrin is similar to that of the other six isomers in cypermethrin;

- The metabolism and radiodepletion studies are not adequate and, therefore, a very conservative estimate of the marker compound as a percentage of total residues in all food species is proposed. The percentages proposed for the estimation in individual tissues of total residues from the parent drug are; muscle, 30; liver, 10; kidney, 5; fat, 60; milk, 80; eggs, 30;

- There is adequate residue information from the non-radiolabelled studies using the recommended formulations; and

- There are analytical methods available, however, evidence of validation is needed.

The Committee recommends temporary MRLs for cattle, sheep and poultry of 100 μg/kg in muscle, liver and kidney, 500 μg/kg in fat, 25 μg/kg for cows whole milk and 50 μg/kg for eggs expressed as parent drug.

An estimate of the residue intake is tabulated as follows:

Tissue	Food Basket (g)	MRL (μg/kg)	μg	Percent UD/TR	Intake (μg)
Muscle	300	100	30	30	100
Liver	100	100	10	10	100
Kidney	50	100	5	5	100
Fat	50	500	25	60	42
Milk	1500	25	37.5	80	47
Eggs	100	50	5	30	17
				Total	406

UD is unchanged drug; TR is total residues.

The above MRLs accommodate the ADI and the recommended use of this compound as a veterinary drug.

The Committee requests the following information:

1. Radiodepletion studies in sheep and poultry which extend beyond the recommended withdrawal times

and using the drug in its topical formulation. The study should determine the depletion of the total residues and the parent drug;

2. The radiodepletion study submitted for cattle should be reassessed to determine the depletion of the total residues and the parent drug;

3. Evidence of lack of interconversion of the cis isomeric forms to the trans forms during metabolism in the target species; and

4. Further information on the validation of the analytical methods; particulary data on the derivation of LOD and LOQs.

REFERENCES

Cameron, D.A., Redgrave, V.A., MacDonald, I.A. and Gillis, N.A., (1993). Alphacypermethrin: Concentrations of Residues in Bovine Tissues. HRC report SLL 279/931913

Crawford, M. J. and Hutson, D. H., (1977). The metabolic fate of WL 43467 (cypermethrin). Metabolism and elimination of ^{14}C-aryl-labelled cis and trans isomers in rats. TLGR 0131.77.

Francis, W.P. and Gill, J.P., (1989). Flufenoxuron/Alphacypermethrin - Residues in Sheep Tissues Following Treatment with Pampass/Renegade Mixtures by Dip or Pour-on.

Morrison, B.J. and Richardson, K.A., (1994). WL 85871 (ACYP, FASTAC) . The metabolism of ^{14}C-WL85871 after Repeated Oral Dosing in the Lactating Cow In-life Phase and Metabolite Profiling.

Redgrave, V.A., Cameron, D.M., Elsom, L.F., Girkin, R. and Cheng, C., (1992). ^{14}C-Alphacypermethrin: Concentrations of Residues in Bovine Milk and Tissues. HRC report SLL 240/920997

SAMS 456-1, (1988). Determination of Residues of Alphacypermethrin in Milk. Gas Liquid Chromatographic Method.

SAMS 461-1, (1988). Determination of Residues of Alphacypermethrin in Animal Tissue. Gas Liquid Chromatographic Method.

Sherren, A.J., (1988a). Residues of Alphacypermethrin in Milk Following Topical Treatment of Cows with "Renegade" Pour-on in the UK.

Sherren, A.J., (1988b). Residues of Alphacypermethrin in Cattle Tissues Following Topical Treatment of Calves with "Renegade" Pour-on in the UK.

Sogeval, (1992). Summary of Residues Trial in Hens and Eggs - Sogeval 1992.

White, D.A., (1987). Report on Residues of Cypermethrin and Alphacypermethrin in Sheep Tissues.

MOXIDECTIN

First draft prepared by
Dr. Raymond J. Heitzman
Compton, Newbury
Berkshire, United Kingdom

ADDENDUM
to the Moxidectin residue monograph prepared by
the 45th meeting of the Committee and published in
FAO Food and Nutrition Paper 41/8, Rome 1996

Introduction

Moxidectin was evaluated at the 45th JECFA and the Committee recommended MRLs for cattle, sheep and deer of 500 μg/kg in fat, 100 μg/kg in liver, 20 μg/kg in muscle and of 50 μg/kg for kidney expressed as parent drug. The MRL for deer were temporary. Since then the sponsors have carried out further analytical work on their large study in sheep which has highlighted that the residues in sheep muscle can exceed the MRL if the recommended dosing schedule for psoroptic mange is used. They determined the residues of moxidectin in muscle, liver, kidney and at the injection sites (data for fat was submitted to 45th meeting) at various times following two injections of moxidectin 1.0% injectable, ten days apart, at the recommended rate of 0.2 mg moxidectin/kg body weight.

Residue Study

Thirty-nine Blackface x Cheviot x Suffolk lambs, aged approximately nine months and weighing 38 to 64 kg on Day 0, were used. Five groups of six lambs, three castrated males and three females each were treated with moxidectin 1.0% injectable solution on Day 0. The remaining animals served as controls or back-up animals. Six treated plus two untreated lambs were euthanized on Day +10 after treatment and samples of back fat, loin muscle, kidney, liver and injection site muscle were removed and frozen for residue analysis. At the same time the remaining twenty-four lambs were given a second injection of moxidectin on the opposite side of the neck. All animals were euthanized in groups of six on Days +20, +30, +40 and +50 respectively. On each slaughter occasion, samples were collected from the same sites as were taken from lambs on Day +10. All the samples collected were divided in half before freezing. At the end of the experiment half were transferred for analysis and the remainder were retained frozen. To accurately quantify the moxidectin residue levels at the injection site, the frozen injection site retention samples were analysed (Parker, 1995). The results for analysis of tissues are summarized in Table 1.

Three main points emerge from this study:

1. The residues in muscle exceed the MRL proposed at the 45th JECFA. The maximum value at any time was 63 μg/kg at 10 days post dosing but thereafter (20-50 days) no value exceeded 40 μg/kg even though two doses were administered;

2. The levels in liver, kidney and fat did not exceed the recommended MRL; and

3. There were very high and persistent residues at the injection sites.

In the submissions to the 45th JECFA residues of moxidectin were not measurable (< 10 μg/kg) in the muscles of sheep by 28 days. However in this study it was clear that residues persisted for at least 50 days. That there are residues in ovine muscle compared with no detectable levels in bovine or deer muscle was probably due to the high fat content in sheep muscle and the lipophilic nature of moxidectin.

Table 1. Residues of Moxidectin (μg/kg) in edible tissues and at the injection sites after one or two injections of moxidectin at a dose of 0.2 mg/kg body weight

Days post dose	Muscle	Liver	Kidney	Fat	Injection Site 1	Injection Site 2
10	16-63 [41±20]	14-36 [21±8]	<10-18 [NA]	167-314 [222]	819-2985 [1582±700]	no 2nd injection
20*	22-40 [29±6]	15-41 [29±8]	11-25 [21±5]	197-433 [324±89]	159-2159 [652±697]	409-3734 [1353±1176]
30*	<10-32 [NA]	<10-25 [NA]	<10-17 [NA]	183-284 [234±41]	202-1345 [551±377]	217-963 [660±234]
40*	<10-15 [NA]	<10-13 [NA]	<10 [NA]	91-223 [139±42]	67-177 [125±41]	106-424 [207±106]
50*	<10-22 [NA]	<10-12 [NA]	<10-16 [NA]	91-290 [164±69]	87-379 [177±96]	79-451 [185±127]

* Sheep received 2nd injection on day 10. NA is not applicable because some values were below the limit of determination of the method. No residues were detected in control tissues.

Maximum Residue Limits

The established ADI is 120 μg for a 60-kg person (45th JECFA). The MRL previously proposed used up 79 μg of this daily allowance. In view of the observed residues in sheep muscle the Committee recommended increasing the MRL specifically for sheep muscle to 50 μg/kg. Using the same factors (i.e. moxidectin accounts for 40% of the residue of concern in muscle) this would result in a theoretical increase from 15 to 37.5 μg of moxidectin equivalents for sheep muscle and the additional intake would not exceed the ADI.

REFERENCE

Parker, L.D., (1995). Residue Depletion Study in sheep following two treatments with moxidectin 1.0% injectable, ten days apart. Report GASD 02-41.00. Cyanamid.

NEOMYCIN

First draft prepared by
Dr. Dieter Arnold
Federal Institute for Health Protection
of Consumers and Veterinary Medicine
Berlin, Germany

ADDENDUM
to the Neomycin residue monograph prepared by
the 43rd meeting of the Committee and published in
FAO Food and Nutrition Paper 41/7, Rome 1995

No new residue depletion studies were submitted by the sponsor. Therefore, no new residue monograph was prepared. However, in view of the final ADI established by the Committee at the present meeting certain residue data were re-evaluated before final MRLs were established. These selected studies support the oral use of neomycin sulfate in certain food animals.

In a series of recently conducted residue depletion studies in cattle, swine, sheep, and goats, respectively, neomycin sulfate equivalent to 15.4 mg of neomycin base per kg of body weight was given to the animals as a single daily dose over fourteen days. No measurable residues of neomycin were found in any of the samples of liver, muscle and fat taken at any time after the last administration of the drug to the animals. The limit of quantification was 0.5 mg/kg in these studies. Kidney was considered to be the target tissue in these species and parent neomycin was established as the marker residue. The range of concentrations of neomycin found in the kidneys of cattle, swine, sheep and goats at one day withdrawal time was from below the limit of detection up to about 4.2 mg/kg.

However, when a separate similar depletion study was conducted in young calves (three days old at the beginning of the study), the depletion of the residues from kidney was slow in these young animals. Twenty-eight days after the administration of the last dose 3.9-6.8 mg of neomycin per kg of tissue were still found in the kidneys of the four animals slaughtered at this sampling time.

In a study conducted in 1967 where 15 female calves of an average body weight of 170 kg had been given oral doses of neomycin sulfate equivalent to 7.7 mg of neomycin base per kilogramm of body weight per day on five consecutive days measurable concentrations of neomycin residues were also found in the livers of some animals (e.g., 2.75 mg/kg at 3 days withdrawal, 1.01 mg/kg at 7 days withdrawal, 0.62 mg/kg at 17 days withdrawal and 1.7 mg/kg at 24 days withdrawal). However, such findings have not been confirmed in the contemporary studies. The Committee concluded that the establishment of MRLs in edible tissues should be based on the results obtained in the well designed and well documented contemporary studies.

Maximum Residue Limits

The Committee, at its 43rd Meeting, had recommended temporary MRLs because the ADI was temporary. These MRLs were: kidney 5 mg/kg, and muscle, liver and fat 0.5 mg/kg expressed as parent drug for cattle, sheep, goats, pigs, turkeys, ducks and chickens. The temporary MRLs recommended for chicken eggs and cow's milk were 0.5 mg/kg and 0.5 mg/l respectively, expressed as the parent drug.

The Committee concluded that it was unnecessary to change these MRLs with the exception of the MRLs for kidney. In the case of kidney the Committee recognized that in order to enable the establishment of practical withdrawal times for all target animal species it was necessary to double the MRLs for kidney from 5 mg/kg to 10 mg/kg.

The following final MRLs were recommended for cattle, sheep, goats, pigs, turkeys, ducks and chickens: kidney 10 mg/kg, and muscle, liver and fat 0.5 mg/kg expressed as parent drug. The final MRLs

recommended for chicken eggs and cow's milk are 0.5 mg/kg and 0.5 mg/l respectively, expressed as the parent drug.

From the above MRL values, the calculated theoretical maximum daily intake of neomycin residues is 1525 micrograms, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, 100 g of eggs and 1.5 l of milk (see the Report of the 34th Session of JECFA). This is considerably less than the maximum ADI of 3600 micrograms of neomycin for a 60-kg person.

OXYTETRACYCLINE

First draft prepared by
Dr. P. Sinhaseni Tantiyaswasdikul
Department of Pharmacology
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Bangkok, Thailand

ADDENDUM
to the Oxytetracycline residue monograph prepared by the 45th meeting of the Committee and published in FAO Food and Nutrition Paper 41/8, Rome 1996

The Committee, at its 45th meeting, stated that a validated analytical method for the determination of oxytetracycline residues in prawn tissue was required for evaluation at the 47th meeting of the Committee before a permanent MRL could be assigned to oxytetracycline in prawn.

A method for the quantification of oxytetracycline in giant prawn was submitted for evaluation at the present meeting by the Department of Medical Sciences, Ministry of Public Health, Thailand. The method has a limit of detection (LOD) of 10 μg/kg and a limit of quantification (LOQ) of 50 μg/kg with a coefficient of variation of 21% at LOQ. The supporting validation data is summarised below.

The oxytetracycline method is based on the Oka method for tetracyclines which is accepted as the benchmark method for this purpose (Oka et al, 1985).

The method for prawn, which is required to monitor a proposed MRL of 100 μg/kg, has been tested in two laboratories utilising slightly different validation regimes. The first laboratory used fortification levels 50, 100 and 200 μg/kg whereas the second laboratory chose fortification levels of 100, 200 and 400 μg/kg. The first laboratory used two separate analysts to produce data on six samples each analysed twice, at the 50 μg/kg fortification level. The results are shown in Table 1.

Table 1. **Analytical Data for Recovery of Oxytetracycline from Prawn Meat at 50% of the MRL (50 μg/kg)**

	% Recovery of Oxytetracycline	
	Analyst 1	Analyst 2
Run 1	52, 55, 67, 60, 77, 47	91, 95, 84, 84, 87, 96
Run 2	96, 119, 110, 80, 84, 82	89, 96, 90, 98, 89, 88
Mean	78	91
SD	23	4.7
%CV	29	5.2

This data gives a mean recovery of 84% with a %CV of 21% at the LOQ which is 50% of the MRL.

In the second laboratory one analyst conducted six recoveries at fortification levels of 100, 200 and 400 μg/kg, each being analysed twice. Results are summarised in Table 2. It should be noted that data for the 50 μg/kg level presented for Laboratory 1 is the same data obtained by two separate analysts presented in Table 1.

Table 2. **Analytical Data for Mean Recovery of Oxytetracycline from Prawn Meat at Different Fortification Levels at or near the MRL by Two Different Laboratories**

Run		Laboratory 1		Laboratory 2	
	Fortification Level (μg/kg)	% Mean Recovery (n=6)	% CV	% Mean Recovery (n=6)	% CV
1	50	60	18	NM	NM
	100	89	9.2	97	5.8
	200	86	10.7	88	4.8
	400	NM	NM	92	12.1
2	50	95	17.1	NM	NM
	100	93	5.4	82	14.3
	200	85	4.6	78	18.1
	400	NM	NM	88	11.4
3	50	89	5.8	NM	NM
	100	92	2.7	NM	NM
	200	91	9.9	NM	NM
	400	NM	NM	NM	NM
4	50	91	5.6	NM	NM
	100	89	1.6	NM	NM
	200	85	2.5	NM	NM
	400	NM	NM	NM	NM

NM = Not Measured

APPRAISAL

A method for the quantitative determination of oxytetracycline in giant prawn was submitted for consideration. The analytical method submitted is Method 995.09 AOAC International, for analysis of residues of chlortetracycline, oxytetracycline and tetracycline in bovine and porcine muscle and kidney. This method was evaluated for use in prawn muscle.

The performance testing results for oxytetracycline in prawn muscle included data from two laboratories and three analysts. The results indicate that the recovery of oxytetracycline from giant prawn muscle at the temporary MRL established at the forty-fifth meeting of the Committee (100 μg/kg) was 82-97%. Recovery at 50 μg/kg was 60-91%. The coefficient of variability for analyst repeatability was acceptable.

These values are similar to recovery reports of other studies in different tissues.

Considering that the requested analytical method has been provided and that it is acceptable, the Committee recommended an MRL of 100 μg/kg for oxytetracycline residues in giant prawn muscle.

REFERENCE

Department of Medical Sciences, Ministry of Public Health, (1996). Validation of the analytical method of oxytetracycline in giant prawn, Thailand, May 1996.

SPIRAMYCIN

First draft prepared by
Dr. B.L. Marshall
New Zealand Embassy
Washington DC., USA

ADDENDUM
to the Spiramycin residue monograph prepared by
the 43rd meeting of the Committee and published in
FAO Food and Nutrition Paper 41/7, Rome 1995

Introduction

As the sponsor was unable to provide a validated chemical method for the analysis of spiramycin and neospiramycin residues in pig tissues to the 43rd JECFA meeting in 1994, it was not possible to estimate the contribution that these residues would make to total residues, and as such the Committee requested that the sponsor provide the following information for consideration by the 47th JECFA meeting in 1996.

1. A validated analytical method for spiramycin and neospiramycin in pig tissues.

2. Residue data to estimate the percentage of the total antimicrobial activity represented by spiramycin and neospiramycin in pig liver, kidney and fat.

RESIDUES IN FOOD AND THEIR EVALUATION

Methods of Analysis for Residues in Tissues

In response to the Committee's request, the sponsor provided performance validation data from a number of authors, to support microbiological agar gel diffusion methods for spiramycin determination. (Pascal et al., 1990b; Cuypers et al., 1994; and Daix and Gougeard, 1996) The sponsor also reviewed two HPLC methods for spiramycin determination in pig liver (Mourier, et. al., 1993; and applied by Cuypers et al., 1994) and in pig muscle and liver tissues (National Agency of Veterinary Medicine - CNEVA, Fougères, France, 1993, that has been implemented by CEPHAC Research Centre, Saint Benoit, France). Reference was also made by the sponsor, to a third HPLC method for pig muscle and liver tissues (Mignot, Lefebre and Millerioux., 1993) but the data was not presented because the results were determined to be ambiguous, unclear and not consistent with those obtained using the microbiological assay method accepted at the 38th JECFA meeting. Not only did the authors record chromatographic interference but the analytical results were apparently significantly lower than those obtained by the microbiological assay. A further HPLC method deemed appropriate by Danish Authorities, for the determination of spiramycin and tylosin residues in pig muscle tissue, was also received for consideration, from Nielson et al., (1995) of the Danish State Veterinary Laboratory.

Data was provided by the sponsors to support the recommendation that the microbiological gel diffusion assay for the determination of spiramycin and its active metabolites, that was developed by Pascal et al. in 1989, submitted to JECFA in 1990, recognised in the European Pharmacopoeia, and further validated by Cuyper et al., 1994, and Daix and Gougeard, 1996, is more appropriate for routine monitoring of pig tissues than HPLC analysis. Comparative studies of the HPLC and microbiological methods by Cuypers et al., confirmed excellent correlation between the two.

The microbiological diffusion assay was performed according to the criteria described in the European Pharmacopoeia, II edition, using *Micrococcus luteus* ATCC 9341 as the test strain and medium A (without pancreatic digest of casein) as culture medium. Ground liver samples were extracted three times with a mixture of methanol and pH 9 phosphate buffer, 7:3. After evaporation of the methanol and adjustment of pH, the solutions were deposited on inoculated culture media, incubated at 30°C for 36 hours, zones of inhibtion read and sample titers calculated.

Validation of Analytical Methods

Microbiological Methods

The Pascal et al., 1990b, residue feeding study was designed to determine the kinetics of spiramycin elimination in muscle, liver, kidney and fat tissues of sixty-six Large White X French Landrace male and female piglets, approximately 11 weeks of age and weighing 25-30 kg, which had been fed daily for 7 days, medicated feed containing 16 or 25 mg/kg BW spiramycin embonate (WHO standard 3200 IU/mg). To determine possible interference on the elimination kinetics of spiramycin treated animals, from concomitant use of oxytetracycline (OTC) or sulphamethazine (SMZ), two of the animal groups, each of 12 animals, were also fed feed containing 32 mg/kg BW OTC or 32 mg/kg BW SMZ. The absence of interference with OTC or SMZ was evaluated by incorporating spiramycin in blended liver or kidney tissues with twice as much OTC or SMZ.

At each evaluation time, 2-4 piglets were slaughtered, and six 8-10 g samples of each edible tissue taken for analysis. After solvent extraction and clean-up, spiramycin was assayed by agar diffusion, using *Micrococcus luteus* ATCC 9341 as the test organism. Reference spiramycin solutions were prepared using spiked tissue. This microbiological assay method was validated in terms of linearity, parallelism; extraction yield; sensitivity of titration; and repeatability.

The analytical parameters of the method, including extraction yields, assay sensitivity and repeatability and the mean coefficient of correlation of liver, kidney and muscle test samples (from triplicate, quintuplicate and a single test sample) are summarised in Table 1.

Table 1. Analytical Parameters of the Pascal et al 1989 Microbiological Assay in Pigs

Tissue	Coefficient of correlation	Extraction yield (%)	Sensitivity (LOD) (μg/kg)	Repeatability (%)
Liver	0.945	at 3000 μg/kg: 80 at 6000 μg/kg: 84	300	1.97 (SD=0.22)
Kidney	0.940	at 1200 μg/kg: 83 at 1600 μg/kg: 79 at 8000 μg/kg: 89	150	1.12 (SD=0.08)
Muscle	0.946	at 500 μg/kg: 90	100	
Fat	0.629	at 500 μg/kg: 69	100	

While the performance standards were generally very acceptable, the coefficient of correlation and the extraction yield for fat was poor. This undoubtedly is due to the greater physical heterogeneity of the tissue and hence the difficulty in homogenising and extracting the residues.

In the study, tissue concentrations in animals receiving 16 mg/kg BW/d spiramycin, rapidly decreased after treatment ceased, regardless of whether spiramycin had been administered in feed separately or in conjunction with OTC or SMZ. The data shows that muscle residue concentrations fell to 120 μg/kg within 12 hours of cessation of treatment and were at or below the limit of detection by day 3. By day 10 liver and kidney concentrations were below the detection limit of the method, 300 and 150 μg/kg, respectively. For animals receiving 25 mg/kg BW/d, liver and kidney concentrations were below the detection limit on day 20, with muscle and fat concentrations being below the limit of detection regardless of the sampling time frame (7,10 and 20 days post-treatment). Spiramycin residue concentrations in fat were found to be consistently below the detection limit (100 μg/kg) of the method at the both dose levels.

The antimicrobial activity of neospiramycin was determined to be closely equivalent to that of spiramycin.

Table 2 summarises the mean triplicate spiramycin concentrations (μg/kg) in liver and kidney tissue samples determined by microbiological assay from piglets fed 16 mg/kg BW spiramycin alone or in conjunction with OTC or SMZ fed at 32 mg/kg BW/d for 7 days.

Table 2. Absence of Interference by OTC or SMZ on the Elimination Kinetics of Spiramycin in Treated Piglets

Tissue	Liver (μg/kg)			Kidney (μg/kg)		
Withdrawal (days)	Spir	Spir+OTC	Spir+SMZ	Spir	Spir+OTC	Spir+SMZ
0	6270			8930		
3	1430			1280		
7	580	640	430	210	240	170
10	<300	380	<300	<150	<150	<150
15	<300	<300	<300	<150	<150	<150
20	<300	<300	<300	<150	<150	<150

Spir - spiramycin; OTC - oxytetracycline; SMZ - sulphamethazine (sulphadimidine)

Mean spiramycin tissue residue recovery values for liver and kidneys are summarised in Table 3.

Table 3. Mean Spiramycin Tissue Recovery Values, Expressed as a Percent with Respect to the Addition of Spiramycin Alone

Tissue	Spiramycin + OTC	Spiramycin + SMZ
Liver	111 (SD = 5.2)	95 (SD = 4.2)
Kidney	93 (SD = 12.2)	102 (SD = 6.6)

Data presented demonstrated that in a study to determine the sensitivity of *M. luteus* ATCC 9341 to OTC alone, no antibiotic activity was evident at concentrations ranging from 50 to 500 μg/l. Similarly there was no significant interference of OTC evident in the assay for spiramycin, although there was a little synergy, either with or without extraction, when OTC was added at twice the concentration of spiramycin.

Table 4 indicates the % recovery with or without extraction, when spiramycin was present in concentrations of 44 to 500 μg/l and OTC, 88 to 1000 μg/l.

Table 4. Absence of Interference of OTC in the Determination of Spiramycin With* or Without** Extraction. Concentration OTC = 2 x Concentration of Spiramycin

	Spiramycin	OTC	Spiramycin+OTC
% Recovery**	100	0	108
% Recovery*	100	0	112

There is little or no change in the elimination kinetics of spiramycin in animals where oxytetracycline or sulphamethazine has been administered in combination with spiramycin, as compared to animals dosed with spiramycin alone and at the same dose rate. Given the precision of this method, it can be concluded that with little interference from OTC or SMZ, this microbiological assay would be suitable for routine monitoring of spiramycin residues in pig tissues.

Another study reported by Daix and Gougeard., 1996, further defined the validation parameters (limits of detection, quantification and repeatability) of the Pascal et al., 1990b microbiological agar diffusion method for pig liver, kidney, muscle and fat tissues and demonstrated the suitability of this method for routine monitoring of pig tissues. Limits of detection and quantification were determined by testing 3 replicates, at each of the 3 concentrations of incurred residues. This involved 1/3 dilutions of the control extracts that had been prepared by adding a quantity of reference spiramycin to 10 g of homogenised tissue. The estimated repeatability was based on testing 6 replicates of each tissue of liver, kidney, muscle and fat, at 2 x MRL, as allocated by the 43rd JECFA, for the corresponding tissue, ie at concentrations corresponding to 2 x 600 μg/kg in liver; 2 x 300 μg/kg in kidney; 2 x 200 μg/kg in muscle; 2 x 200 μg/kg in fat. The relative standard deviation for the inhibition zone was found to be less than 5% for all tissues. Results of this study are summarised in Table 5.

Table 5. Detection, Quantification and Repeatability Validation Parameters for the Spiramycin Microbiological Assay of Different Pig Tissues

Tissue	Limit of Detection (μg/kg)	Limit of Quantification (μg/kg)	Repeatability (%)
Liver	140	300	2.9
Kidney	140	300	2.9
Muscle	45	100	2.5
Fat	70	115	2.2

It was concluded that the microbiological diffusion assay developed by Pascal et al., (1990b) and described in report RPS JP/LY ref. 1103 of January 1990, has a satisfactory repeatability in the four tissues studied and at concentrations consistent with practical conditions.

Chemical Methods (HPLC)

An HPLC method developed by the National Agency of Veterinary Medicine - CNEVA, Fougères, France, for the determination of spiramycin and neospiramycin in cattle tissues and modified by Mignot et al (1993), was evaluated as to suitability for routine screening or as a reference method, for pig liver and muscle tissues. Both spiramycin and neospiramycin were extracted from tissues by liquid-liquid extraction, followed by solid-liquid

phase extraction. The eluates were chromatographed using reverse phase high performance liquid chromatography (HPLC) with an acidic mobile phase and UV detection at 231 nm. The method was fully validated in muscle for spiramycin and neospiramycin, in terms of linearity, recovery, with intraday and interday (frozen muscle samples) precision and accuracy being considered acceptable. CV and % error were both <20%, and the LOQ was validated at 25 μg/kg for both compounds. The mean recovery of the associated quality control freshly prepared samples was equal to 93% for spiramycin and 100% for neospiramycin. Only 4 out of 16 samples however, had residue concentrations above the limit of quantification. While the liver tissue assay was validated in terms of linearity and intraday precision and accuracy, the recovery was <50%, and the interday precision and accuracy could not be determined. Although spiramycin and neospiramycin residues were quantified in liver tissue samples with incurred residues, and a mean recovery of 83% for spiramycin and 80% for neospiramycin determined, residue concentrations above the limits of quantification 200 μg/kg for spiramycin and 100 μg/kg for neospiramycin were measured in only 5 out of 16 samples. Due to chromatographic interferences in liver and residues invariably being below the limit of quantification in muscle and liver tissues, it was concluded that this procedure would not be suitable for routine analysis of pig muscle and liver tissues.

An HPLC method, developed by the Food Control Laboratory of the Danish Veterinary Service (Petersen, et al., 1995) and currently used in Denmark to determine spiramycin and tylosin residues in muscle tissue, was forwarded to JECFA for consideration. The data demonstrated that while the method is suitable for muscle tissue as a screening or reference method, it is unsuitable for kidney or liver tissues. While plasma analysis is considered possible, the laboratory was unable to provide documentary evidence on the ratio distribution between plasma and muscle tissue. The results of 16 blind pig muscle samples used to determine detection (LOD) and quantification (LOQ) limits for spiramycin and tylosin are shown in Table 6.

Table 6. LOD and LOQ of Spiramycin and Tylosin in Pig Muscle

Analyte	LOD (μg/kg)	LOQ (μg/kg)
Spiramycin	18	33
Tylosin	27	40

Repeatability and reproducibility, done on spiked standard curves run twice a day over six days, are shown to be high at 0 μg/kg for spiramycin and tylosin. The reason is, that noise on the base line varies both under clean-up at day one, and between days. All peaks were integrated using the same method of integration, with peaks with 0 μg/kg values, not being subject to forced integration. A low pH was found to be necessary during optimisation of the extraction process in order to obtain a good recovery for spiramycin, however, a low pH could give a lower recovery for tylosin. An assessment of the robustness and extraction process showed that while the quantity of solvent used was significant on the percentage recovery of spiramycin, interactions were not found to be significant.

From the data provided, not only do liver concentrations also need to be considered cautiously, because of chromatographic interferences, but spiramycin and neospiramycin concentrations in pig liver and muscle are often below the limit of quantification. The method should only be considered useful for determining the total spiramycin and tylosin residue concentrations in muscle tissues.

Percentage Total Antimicrobial Activity Represented by Spiramycin and Neospiramycin in Pig Liver

An HPLC method, developed by Mourier (1993), and applied by Cuypers et al (1994), was used in a study, in conjunction with a microbiological assay, to determine the metabolism of spiramycin (embolate) in liver tissues of 12 treated pigs, fed 22 mg/kg bw/d spiramycin in feed for 7 days, 4 of which were slaughtered at each experimental withdrawal time points of zero, 3 and 10 days. The percentage of total activity represented

by spiramycin and neospiramycin in pig liver was also determined. The method incorporates an internal standard RP 22711, a column temperature set at 60°C and involves extraction with acetonitrile:water (90:10, v/v), which can detect transformed spiramycin down to 200 μg/kg. Concentration of the extract on a solid phase extraction precolumn occurs before separation and HPLC analysis using UV detection at 232 nm, the absorption maximum of spiramycin.

Table 7 indicates the extraction titers data obtained from Mourier's acetonitrile/water extraction, incurred residue control study. Spiramycin I and III accounted for 400 μg/kg out of the total antimicrobial activity of 13800 μg/kg. The extraction titer for the internal standard was 21500 μg/kg. The percentage of total microbial activity represented by spiramycin (I and III) and neospiramycin in this incurred pig liver study was calculated to be 2.8%.

Table 7. Extraction Tilers (μg/kg) of Spiramycin and Its Metabolites from Treated Pig Liver

	tr Neospira I	tr Spira I	Spira I	tr Neospira III	tr Spira III	Spira III
(μg/kg)	1400	6600	200	900	4500	200

tr = cysteine conjugate; Neospira = neospiramycin; Spira = spiramycin

As the first extraction is not complete, an underestimation could occur of the quantity of transformed spiramycin and transformed neospiramycin because of their higher polarity.

The report of Cuypers et al., (1994) demonstrates (Table 8) mean spiramycin residues levels (μg/kg) in liver tissue of pigs fed daily 22 mg/kg bw spiramycin medicated feed for 7 days.

Table 8. Mean Spiramycin Metabolite Residue Levels (μg/kg) in Liver Tissue of Pigs Fed 22 mg/kg/d Spiramycin Medicated Feed for 7 Days

	Spiramycin metabolites (μg/kg)*					
Withdrawal (days)	tr Neospira I	tr Spira I	Neospira I	tr Neospira III + Spira I (coelution)	tr Spira III	Spira III
0	200	1800	100	1000	1800	200
3	ND	80	130	430	280	ND
10	ND	ND	30	ND	80	ND

*Mean of four pigs; results expressed in μg/kg calculated with reference to internal standard RP 22711; ND = Not Detected; limit of quantification 100 μg/kg

The conclusion of these studies was that the parent drug spiramycin, which consists of two major components, spiramycin (I and III), is found in liver extracts of orally treated pig, in the three forms, L-cysteine transformed spiramycin and neospiramycin, and non transformed spiramycin base, generally present in very small quantities. The transformed/non-transformed base ratio is never found to be less than 0.9, and depends above all, on the quantity of the L-cysteine present in the extract. The ratio of Spiramycin I^{Liver} (tr spiramycin I + tr neospiamycin I+ spiramycin I)/Spiramycin III^{Liver} was calculated to be around 1.5, about ten times lower than the Spiramycin I/Spiramycin III ratio characteristic of spiramycin used for treatment. Spiramycin II, present

in feed in negligible quantities was not found in liver in any form whatsoever. The internal standard (RP 22711) is not transformed by L-cysteine, but found in liver in its original form. Control liver showed no trace of spiramycin or its derivatives. Both the repeatability and reproducibility of spiked control liver were considered satisfactory. Table 9 and the 2.5% CV of reproducibility of the internal standard, indicate that extraction and injection are reproducible.

Table 18. Percentage Coefficient of Variation of Repeatability and Reproducibility of Control Spiked Liver

Repeatability

	tr Neospira I	tr Spira I	Neospira I	tr Neospira III + Spira I Coelution	tr Spira III	Spira III
CV (%)	7.3	6.5	21.3	11.7	2.6	12.2

Reproducibility

	tr Neospira I	tr Spira I	Neospira I	tr Neospira III + Spira I Coelution	tr Spira III	Spira III
CV (%)	30.2	6.1	16.3	10.0	4.6	24.4

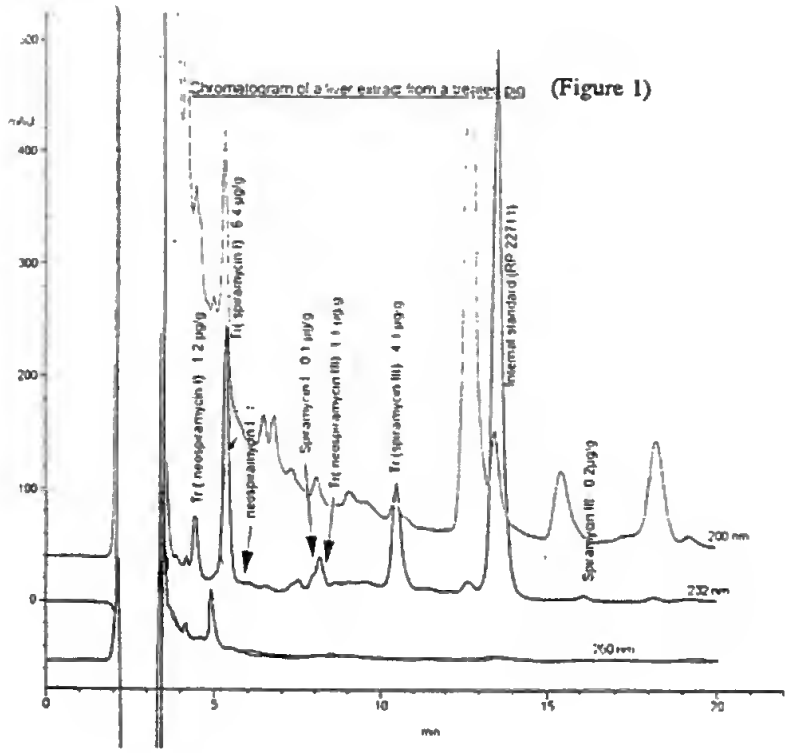
tr = L-cysteine conjugate

Approximate retention times are provided in Table 10. A chromatograph of a liver extract from a treated pig, demonstrating the complexity involved in quantifying spiramycin residues by HPLC, is provided in Figure 1.

Table 10. Approximate Retention Times (min) of Spiramycin and its Derivatives

Solute	Retention Time Relative to Spiramycin I	Retention Time (min)
tr Neospiramycin I	0.56	4.3
tr Spiramycin I	0.67	5.2
Neospiramycin I	0.74	5.7
Spiramycin I	1.00	7.7
tr Spiramycin III	1.30	10.1
Internal Standard RP 22711	1.71	13.2
Spiramycin III	2.00	15.5

tr = L-cysteine conjugate



(Figure 1)

The microbiological *M. luteus* ATCC 9341 agar gel diffusion assay, described in the European Pharmacopoeia, II Edition and reported in the note of Pascal, 1990, and which was also carried out on the same liver sample as with the HPLC method, showed that there is excellent correlation between the two techniques (Table 11). Microbial values were expressed as spiramycin equivalent. From the data of Mourier et al, 1993, the sum of the metabolites assayed by HPLC were 13800 µg/kg and 12600 µg/kg for the microbial method, proving an excellent correlation.

Table 11. Comparison of Mean Results of HPLC and Microbiological Assays of Quadruplicate Pig Liver Samples

Withdrawal Time (days)	HPLC (µg/kg)	Microbiological Assay (µg/kg)
0	5000 (SD = 1.7)	5300 (SD = 1.6)
3	900 (SD = 0.3)	1300 (SD = 0.1)
10	100	200

A comparison between spiramycin I, II, and III and neospiramycin I and their combinations with cysteine in Mueller-Hinton agar and broth demonstrated that the minimum inhibitory concentration (MIC) or microbiological activities of cysteine conjugates was found to be somewhat lower than the relevant parent compound, with the percentage being 50-100%. Besides with tylosin, a degree of antibacterial cross reactivity has been demonstrated to occur in vitro activities against *Micrococcus luteus* ATCC 9341. (Table 12).

Table 12. *Micrococcus luteus* Inhibition by Four Antibiotics

Antibiotics	MIC (mg/l)
Amoxicillin	0.008
Gentamicin	1
Spiramycin	0.5
Rifamycin	0.008

APPRAISAL

Spiramycin is a macrolide antibiotic that is produced by certain strains of *Streptomyces ambofaciens* and used in oral or parenteral formulations for the treatment or prophylaxis of local or systemic diseases in cattle and pigs. It had been previously evaluated at the thirty-eigbth and forty-third meetings of the Committee. A validated chemical method bad not been available for the analysis of spiramycin and neospiramycin residues in pig tissues, and as sucb it had not been possible to estimate the percentage contribution that these residues would make to total residues.

The 43rd Meeting of the Committee required the following information for evaluation in 1996:

1. A validated analytical method for determining the concentrations of spiramycin and neospiramycin in the edible tissues of pigs.

2. Residue data to estimate the percentage of the total antimicrobial activity accounted for by spiramycin and neospiramycin in the liver, kidney and fat of pigs.

Analytical methods

Microbiological and HPLC methods and respective study data were provided for the determination of spiramycin and neospiramycin in pig tissues.

Microbiological Methods

A method that was submitted for consideration at the forty-third meeting of the Committee was re-evaluated because additional information on the limit of detection, the limit of quantification and repeatability was provided. The method, which has been published in the European Pharmacopoeia, was accepted at the forty-third meeting of the Committee for screening muscle tissues for evidence of spiramycin residues. This method uses a solvent extraction step followed by a microbiological gel diffusion assay involving *Micrococcus luteus* ATCC 9341 as the test strain. The limits of quantification of the method, which were estimated using fortified tissues of a control pig, were 300 μg/kg for liver and kidney, 100 μg/kg for muscle and 115 μg/kg for fat. Recoveries were >80% for liver, kidney and muscle but were lower (69%) for fat. Using incurred tissues at the recommended MRL, the coefficient of variation of the mean of 2-3 replicate analyses of the same sample was in the order of 20-25%, with a range less than 5% to more than 50%. Analyses of incurred liver tissue with residues slightly above the MRL showed that the results with the microbiological assay were, on average, about 40% higher than those obtained with an HPLC method.

The microbiological method was specific for spiramycin in the presence of oxytetracycline and sulfadimidine, which may be formulated in feed together with spiramycin.

The Committee concluded that this microbiological method is suitable to screen pig tissues for residues of spiramycin and its active metabolites, providing that results could be confirmed with a more specific method.

However, the possibility of cross reactivity with hydrophilic antibiotics cannot be excluded.

Chemical methods

Data submitted from a number of HPLC studies were considered. An HPLC method using fortified samples demonstrated suitable sensitivity (limit of detection of 18 μg/kg, and limit of quantification of 33 μg/kg) for analyzing muscle tissue for spiramycin. Due to chromatographic interferences, it was found to be unsuitable for analysis of kidney or liver tissue. It was also specific in the presence of tylosin.

A further HPLC study using spiked samples demonstrated that the limits of quantification for spiramycin and neospiramycin in muscle samples were 25 μg/kg with recoveries of 93 and 100%, respectively. The limit of quantification for liver was 200 μg/kg for spiramycin and 100 μg/kg for neospiramycin, with recoveries in the range of 83 to 80%, respectively.

There was good correlation between the HPLC method and the microbiological assay as demonstrated in the measurement of incurred liver tissues containing residues in the range 100-5000 μg/kg.

The Committee concluded that there are HPLC methods that were suitable for measuring spiramycin residues in muscle, liver and kidney at the level of the MRL. Tylosin did not interfere in the HPLC assay.

Antimicrobial Activity

In an HPLC study, using tissues from pigs fed spiramycin, the spiramycin and neospiramycin cysteine conjugates were found to account for 97.5 % of the residues, with the cysteine conjugates accounting for approximately 90% of the antimicrobial activity of the parent drug, thereby supporting ths use of the antimicrobial assay for routine screening.

Maximum Residue Limits

To promote method validation the Expert Committee considered it appropriate to harmonize MRLs for parent spiramycin residues in different tissues of different food producing animals. The following MRLs were established:

Muscle	(cattle, pigs, chickens)	200 μg/kg
Liver	(cattle, pigs, chickens)	600 μg/kg
Kidney	(cattle, pigs)	300 μg/kg
	(chickens)	800 μg/kg
Fat	(cattle, pigs, chickens)	300 μg/kg
Milk	(cattle)	100 μg/l

expressed as the sum of spiramycin and neospiramycin for cattle and chickens, and as spiramycin equivalents (antimicrobially active residues) for pig tissues.

The Committee agreed that an MRL for chicken kidney should be recommended at 800 μg/kg. Considering a standard daily intake of 300 g muscle, 100 g liver, 50 g kidney, 50 g fat and 1.5 litres milk, a theoretical maximum daily intake of spiramycin residues will be 440 μg. Using an ADI of 0-50 μg per kg of body weight, a 60-kg person, established at the 43rd meeting of the Committee, would therefore be permitted to consume 3000 μg/kg.

The Committee recommended that the current temporary MRLs for pig liver, kidney, and fat be established as full MRLs.

REFERENCES

CNEVA - Fougères HPLC method: from "Deterimination of spiramycin and neospiramycin in biological samples (muscles and liver) from a metabolism study of spiramycin in the pig". Report CEPHAC No. CD 514, April 1993.

Cuypers, V., Mourier, P., Poirier, J. and Rombi, J. (1994). Spiramycin embonate (RP 5337, Embonate). Study of metabolism in pigs. Rhône-Poulenc Rorer Report RPR/RD/CRVA/AN No. 7015, 19 January 1994.

Daix, V. and Gougeard, N. (1996). Spiramycin assay in swine tissues complement of validation of the microbiological assay method. Report RPR/RD/CPD/PQA/CRVA 7370, 15 May 1996.

Ferriot, A. and Videau, D. (1971). Elimination et fixation tissulaire de la Spiramycine chez le porc. Can. Med. Vet., 40, 164-169, 1971.

Huet, A. M., Bosc, F. and Floch, R. (1988). Procedure for the determination of spiramycin in the serum or plasma by microbiological method of diffusion on agar. Rhône-Mérieux Report No. MET 011. Submitted by Rhône-Poulenc Santé, Vitry-sur-Seine, France.

May & Baker (1967). Huntington Research Center, August 1967. Submitted by Rhône-Poulenc Santé, Vitry-sur-Seine, France.

Mignot, A., Lefebre, M. A. and Millerioux, L. (1993). Determination of spiramycin and neospiramycin in biological samples (muscle and liver) from a metabolism study of spiramycin in the pig. CEPHAC Report No. CD 514, April 1993. (p. 44-132).

Mourier, P. (1993). Spiramycin embonate in pigs: study of metabolism. Submitted by Rhône-Poulenc Rorer Report RPR/RD/CRVA/AN No. 6993, 8 December 1993, p. 133-168.

Mourot, D. and Sanders, P. (1988). Determination of spiramycin in the plasma by high performance liquid chromatography. (Ref. Rhône-Mérieux: MET 008) National Veterinary Drugs Laboratory (Fougères). Submitted by Rhône-Poulenc Santé, Vitry-sur-Seine, France.

Nielsen, M. B., Rasmussen, A., Petersen, I., Ørntoft, I. and Petersen, H. W. (1995). Determination of spiramycin and tylosin in muscle - Documentation. Report Submitted by the Ministry of Agriculture and Fisheries, Danish Veterinary Services, Food Control Laboratory, September 1995.

Pascal, C., Bertrand, A., Kies, A. and Poirier, J. (1990b). Spiramycin - Study of residues in swine following administration in feed. Unpublished Report No. 1103. Submitted by Rhône-Poulenc Santé, Vitry-sur-Seine, France, 11 January 1990, p. 1-42.

THIAMPHENICOL

First draft prepared by
Philip G. Francis
Russet House, Shere Road
West Horsley
Surrey, KT24 6EW, England

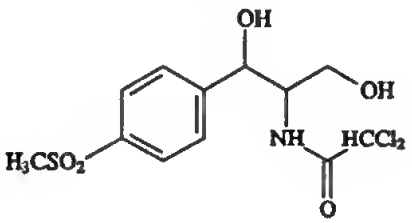
IDENTITY

Chemical names: D-d-threo-2-dichloroacetamido-1-(4-methylsulfonylphenyl)-1,3-propanediol; D(+)-threo-1-(4'-methylsulphonylphenyl)-2-dichloroacetamide propane-1,3-diol; D-threo-2,2-dichloro-N-β-hydroxy-a-(hydroxymethyl)-p-(methylsulphonyl)-phenethyl acetamide (C.A.S. name)

C.A.S number: 15318-45-3

Synonyms: Dextrosulphenidol, Thiophenicol, Win 5063-2, CV8053

Structural formula:



Molecular formula: $C_{12}H_{15}Cl_2NO_5S$

Molecular weight: 356.23

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Appearance: White crystalline powder

Melting point: 164-166°C

Soluhility: Water 0.5%, very soluble in dimethylacetamide (1:1), freely soluble in dimethylformamide and acetonitrile (1:10), soluble in methanol (1:20), slightly soluble in 95% ethanol (1:40) and in acetone (1:50), and barely soluble (1:1000) in ether, ethyl acetate and chloroform

pH (0.5% solution): 5.9. Changes in pH in the range 3 to 9 do not result in significant changes in solubility, but solubility is increased in strongly acid media

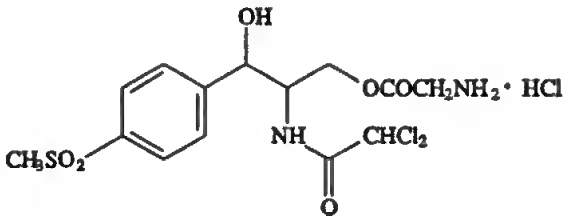
Stahility: Dates of use and batch expiry dates given by contractors producing data for this assessment suggest a shelf life of 5 to 6 years, but no specific statements or recommendations are made by the sponsor, other than a statement that the product is stahle if stored in closed containers, and protected from humidity and excessive heat.

Thiamphenicol glycinate hydrochloride

Thiamphenicol glycinate hydrochloride is the form in which the drug is used for parenteral administration.

C.A.S. number: 2611-61-2

Structural formula:



Molecular formula: $C_{14}H_{19}Cl_3N_2O_6S$

Molecular weight: 449.7

Thiamphenicol content: 79.2%

Solubility: Very soluble in water

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Thiamphenicol is an antimicrobial substance intended for the treatment of infectious diseases in cattle, pigs and poultry. It is used as the water soluble thiamphenicol glycine hydrochloride for parenteral therapy and as a premix composed of thiamphenicol base and corn starch, (4:1) or other mixer, for oral use.

Thiamphenicol has a similar antibacterial spectrum to chloramphenicol (Van Beers et al 1975, Sutter and Finegold, 1976). It has not been associated with aplastic anaemia in spite of extensive use in man (Yunis et al 1973).

Thiamphenicol inhibits protein synthesis in bacteria. It has a bacteriostatic action against a broad range of micro-organisms, although it may be bactericidal for some species under some conditions, and in concentrations 3 to 5 times higher than the bacteriostatic concentrations (Martindale 1971, 1973). Among the bacteria inhibited in vitro by relatively low concentrations of thiamphenicol are Clostridium, Corynebacterium diphtheriae, Diplococcus pneumoniae, Staphylococcus alhus, Streptococcus pyogenes, Streptococcus viridans, Bacteroides, Fusobacterium, Bordatella, Brucella, Haemophilus, Neisseria, Pasteurella, Shigella and some vibrio strains. Some Bacilli, Erysipelothrix, Staphylococcus aureus and Streptococcus faecalis are sensitive to moderate concentrations of thiamphenicol but Listeria, Aerobacter, Escherichia, Klebsiella, Proteus and Salmonellae are sensitive only to relatively high concentrations. The compound is active against Mycoplasmas, Treponema, Rickettsias, Entamoeba and Actinomycetes, but inactive against Mycobacterium tuberculosis and Pseudomonas aeruginosa (Ravizzola et al 1984). The in vitro antimicrobial activity of the thiamphenicol glycinate ester is similar to that of thiamphenicol base.

MIC studies using standard dilution methods were carried out by the sponsor in 1989 and show MIC_{50}-values which are broadly similar to those described above, and by O'Grady et al (1980), but a few strains of

Bacteroides, Escherichia coli, Salmonellae, Staphylococci, and Pasteurellae show high MICs in vitro.

As a summary thiamphenicol is a broad-spectrum antibiotic, active against both Gram-positive and Gram-negative bacteria and especially effective against anaerobes. Thiamphenicol may be used in the treatment and control of a wide range of respiratory and alimentary tract infections of bacterial origin in calves, pigs and poultry. The oral product is not suitable for the treatment of cattle with functional rumen.

Dosage

There appears to be no firm recommendation of dosages in the dossier. Both 30 and 60 mg/kg have been used for calves, 20 - 40 mg/kg for pigs, 15 to 67 mg/kg for poultry, and 30 mg/kg in dairy cows. The oral preparations are not for use in ruminating animals. Administration for sucking calves includes 30 mg/kg of body weight daily of active ingredient, rate of addition to feed being 1000-1500 g/100 kg of milk powder, for pigs 20-30 mg/kg of body weight daily of active ingredient, rate of addition to feed 300-450 g/100 kg and for poultry, rate of addition to feed 400 g/100 kg and to water 200 g/100 l.

METABOLISM

Pharmacokinetics

General

Limited data in the sponsor's dossier show that after an intramuscular dose of 30 mg/kg, thiamphenicol occurs in cattle plasma and cows'milk within 1 to 3 hours of dosing. Similarly after intramuscular dosing the turkey and horse with 100 mg/kg, and the cow with 30 mg/kg, and orally dosing the rat, rabbit and turkey with 100 mg/kg, and the calf with 50 mg/kg, appreciable drug levels occur in plasma, as summarised in Table 1.

Rat

Intravenous administration of chloramphenicol and thiamphenicol to rats at 30 mg/kg showed that the half-life of chloramphenicol was 21.5 minutes whilst that of thiamphenicol was 46.3 minutes. When 80 mg/kg of phenobarbitone was given daily to rats for three days prior to an intravenous dose of thiamphenicol and chloramphenicol (Palmer et al 1972), the half-life of thiamphenicol was unchanged, whilst the half-life of chloramphenicol was reduced by about 50%. This demonstrates that following stimulation of the glucuronyl transferase activity of the liver, the metabolism of chloramphenicol was accelerated whilst that of thiamphenicol was little changed. Liver damage induced by surgery, slowed the metabolism of chloramphenicol but left that of thiamphenicol unchanged. Anuria immediately following anaesthesia in the rat, increased the half life of thiamphenicol, suggesting that the kidney is the main excretory route for the drug. The literature states (Walter et al 1975) that in man, renal insufficiency as measured by creatinine clearance prolonged the half-life of thiamphenicol, but hepatic insufficiency, particularly cirrhosis, did not increase it's half-life. Following oral and intramuscular administration of thiamphenicol to rats at 30 mg/kg and sequential sampling of urine and blood, the GC analysis before and after incubation of samples in the presence of beta-glucuronidase showed that thiamphenicol was excreted in the urine largely in unchanged form. Sampling was terminated after 48 hours, at which time 62% of the oral dose, and 50% of the intramuscular dose had been recovered. In similar experiments in which rats were orally dosed with ^{14}C-thiamphenicol at 30 mg/kg, 62% of the dose was recovered from urine and 35% in faeces within 48 hours after dosing. Two studies in which rats were given a single oral dose of 30 mg/kg of either thiamphenicol or ^{14}C-thiamphenicol gave similar post dose plasma concentrations as shown in Table 2. Radiolabelled thiamphenicol was determined by liquid scintillation counting and unlabelled thiamphenicol by a colorimetric method of McChesney et al, 1960.

Table 1. Thiamphenicol levels (μg/ml) in plasma (Pl) of cattle, turkey, horse, rabbit, and rat and cow's milk (Mk) following a single dose

Hours post dose	Cow 30 mg/kg IM		Calf 50 mg/kg Oral	Turkey 100 mg/kg IM		Horse 100 mg/kg IM	Rabbit 100 mg/kg Oral	Rat 100 mg/kg Oral
	Pl	Mk		IM	Oral			
1					40.3			32.1
2	22.2		17.1		37.0	4.5	6.8	30.6
3		5.7						
4	12.3		13.2	22.6		6.0	4.7	8.5
6	4.0	4.2	3.6		9.7	3.7	2.0	
8	1.7		1.9	8.3			0.8	
9		1.7						
10			0.4		7.7			3.7
11				5.1			0.5	
12	nil	0.6					0.3	
14					3.0			
15		0.1						
16			nil					
18					0.8			
24		nil		nil			nil	nil

Table 2. Plasma concentrations in rat (μg/ml) of thiamphenicol following a single oral dose of 30 mg/kg of radiolabelled and unlabelled drug

Hours post dose	^{14}C-thiamphenicol	Unlabelled drug
2	6.0	5.1 ± 0.8
4	2.3	2.8 ± 0.4
8	1.5	1.6 ± 0.3
24	0.7	0.8 ± 0.2
48	0.7	

Cannulation of the rat bile duct before thiamphenicol dosing, showed that 4% of the dose administered was excreted as unchanged drug in the bile within 4 hours of dosing, and after hydrolysis with beta-glucuronidase

around 12% was shown to be excreted in conjugated form. In other species, <5% of the dose was exreted as glucuronate, and other metabolites accounted for 1-2% of the dose. None of the metabolites were antimicrobial.

Oral dosing of rats at 30 mg/kg with ^{14}C-thiamphenicol indicated that 35% of the administered dose was recovered in faeces and 62% in urine by 48 hours after dosing. Liver, kidney and lung were the organs showing high initial and persisting drug levels.

Whole body autoradiographic studies were carried out in orally dosed rats using ^{14}C-thiamphenicol at a dose of 30 mg/kg. At 2, 4, 8, 24, 48, and 72 hours after dosing, the rats were killed, embedded in carboxymethyl cellulose, frozen, then cut with a microtome to produce 20 μ thick sections. The sections were applied to X-ray films for 20 days. The dossier states that 48 hours after dosing, the highest levels of radioactivity were present in the liver and kidney, with appreciable levels also being present in the thyroid, pancreas, lung, spleen and thymus. Gas chromatography-mass spectrometry analyses demonstrate that thiamphenicol is excreted mainly unchanged, in the urine, although some 1.5% was present as unidentified metabolites. In vitro experiments using ^{14}C-glucuronyl transferase, demonstrate that glucuronidation is not a major part of the metabolism of thiamphenicol, except in the pig, and by inference thiamphenicol does not interfere with the action of chloramphenicol. In vitro studies showed that serum binding of thiamphenicol to human and rat serum is less than 25% (compared with approaching 60% for chloramphenicol.)

Table 3. **Plasma levels of thiamphenicol (μg/ml) in calves given 25 mg/kg orally, twice daily for four days**

Animal	Time from last dose				
Number	6 hours	10 hours	24 hours	28 hours	34 hours
V1	8.63	6.3	1.68	0.60	0.15
V2	9.87	8.9	4.37	2.43	1.10
V4	10.8	7.9	2.04	1.18	0.51
V5	6.2	5.8	1.30	0.51	0.15
V6	8.6	8.1	1.90	1.0	0.35
V7	6.2	6.1	3.28	1.65	0.86
V8	5.6	5.4	2.40	1.0	0.55
V9	5.3	4.3	2.40	1.10	0.60
V10	6.8	5.9	2.40	1.33	0.90
V11	8.1	5.1	1.6	0.51	1.45
V12	4.3	3.3	2.5	1.06	0.23
V13	7.4	5.0	1.0	0.35	0.10
V14	3.4	2.0	0.6	0.30	0.40
V15	6.2	5.3	3.13	1.26	0.10
V16	8.96	8.5	3.65	1.37	0.70
Mean	7.1	5.86	2.25	1.04	0.54
±SD	2.1	1.9	1.05	0.55	0.40

Cattle

Sixteen calves (ages not specified) were orally dosed twice daily with thiamphenicol at 25 mg/kg for four consecutive days. Blood samples were collected for analysis at 6, 10, 24, 28 and 34 hours after the last dose. Calves were killed on the 4th, 6th, 8th, and 10th days after the last dose, and muscle, heart, liver, kidney, spleen, lung, and brain were sampled. Thiamphenicol was extracted with ethyl acetate and potassium carbonate, as described by Bories & Wal, 1983, and analysed by HPLC. Results showed that thiamphenicol concentrations above the LOQ were present in plasma at 34 hours post the last dose, Table 3. Liver, lung, and spleen showed appreciable concentrations for longer than other tissues, but all were below the LOQ eight days after cessation of dosing, Table 4. The extraction efficiency of the method is stated to be 60% and LOQ 20 μg/kg. The extraction efficiency of 67.6% in calf muscle has been determined by Nagata and Saeki (1992) using a similar method to that used by the sponsor.

Table 4. **Tissue levels of thiamphenicol (μg/kg) in calves orally dosed for four days at 25 mg/kg bw per day**

Days after last dose	Animal No.	Tissue						
		Lung	Liver	Kidney	Spleen	Muscle	Heart	Brain
4	2	45	65	50	100	0	130	0
4	10	61	, 77	115	61	0	70	0
4	15	53	65	65	90	0	0	0
6	5	70	<20	0	40	0	0	0
6	7	90	75	120	90	90	110	40
6	9	85	35	0	20	0	0	0
6	11	60	20	0	35	0	0	0
8	1	0	<20	<20	0	0	0	0
8	8	0	0	0	0	0	0	0
8	12	0	0	0	0	0	0	0
8	16	0	0	0	0	0	0	0
10	4	0	0	0	0	0	0	0
10	6	0	0	0	0	0	0	0
10	13	0	0	0	0	0	0	0
10	14	0	0	0	0	0	0	0

Eight lactating cows were given thiamphenicol by intramuscular injection twice daily for 5 consecutive days. The test substance was thiamphenicol glycinate hydrochloride, and the dose was calculated to be 15 mg/kg. Each dose was divided into two equal volumes and administered in different sites at each dosing point. Blood samples were obtained pre-dose and at 0.5, 1, 2, 4 and 6 hours after the first dose. GC with ECD was used to determine thiamphenicol concentrations. The drug appeared to be well tolerated by the animals. Plasma thiamphenicol levels reached about 18 μg/ml by 0.5 hours after the first dose, and were still quantifiable

(2.5 μg/ml) by six hours after the first dose.

Pigs

Three groups each of five pigs (average weight 30 kg) were orally dosed with thiamphenicol every 12 hours at doses of 10, 15 and 20 mg/kg for 5 days. Blood samples were taken at 0, 1, 2, 3, 4, 6, 8, 60, 84, and 108 hours after the first dose and 24 and 48 hours after the last dose for quantification of thiamphenicol and it's glucuronide in plasma using GC with electron capture.

Table 5. **Mean thiamphenicol levels (mg/kg) in pig plasma following feeding with a supplemented diet, equivalent to 30 mg/kg bw/day for five days**

Study day	Time (h)	Thiamphenicol residues
1	0	ND
1	2	1.25
1	4	1.25
1	6	0.85
1	8	1.28
1	16	0.8
2	24	0.24
3	24	0.34
4	24	0.31
5	24	0.22
6	24	0.22
6	4	0.08
6	8	0.05
6	12	0.04
6	16	0.02
6	20	0.02
7	24	0.02
7	12	0.02
8	24	ND
8	12	ND
9	24	ND
9	12	0.02
10	24	ND

ND = Not detected

The maximum plasma concentration of thiamphenicol occurred 1-2 hours after dosing (1.29±0.79, 2.02±0.44 and 2.81±1.86 mg/l, respectively) and was dose related, but no such relationship was evident with thiamphenicol glucuronide. No statistical difference between the $t_{\frac{1}{2}}$ of unchanged thiamphenicol (1.2 hours) and that of it's glucuronide (1.2-1.6 hours) was found. Forty eight hours after the last 20 mg/kg treatment, the mean plasma concentration of unchanged drug was equal to the LOQ (20 μg/l) and twice the LOQ (40 μg/l) 48 hours after the last 40 mg/kg dose. The mean plasma thiamphenicol glucuronide concentrations at all time points after the last dose were higher than the concentrations of unchanged thiamphenicol. No accumulation of drug occurred in plasma during the course of the study.

A further study involved four groups each of 4 pigs with three control animals, weighing 15-22 kg. Animals were fed a cereal diet supplemented with thiamphenicol at 900 mg/kg, equivalent to 30 mg/kg/day, for five days. Blood samples to determine plasma drug concentration were collected at 0, 2, 6, 8, 16, and 24 hours from the start of the trial, at 24 hourly intervals on days 2, 3, 4, 5, and 6 of the trial and 12 hourly thereafter. Analysis was carried out by HPLC. Peak thiamphenicol levels in plasma (1280 μg/kg) were found 8 hours after the first dose, with mean concentrations of 220-800 μg/kg being found during the remainder of the dosing period. Levels declined to the LOQ (20 μg/kg) by 48 hours after the last dose. The extraction efficiency for plasma was 92.6 ± 10.2%, Table 5.

Sheep

Twelve sheep, 9-12 months of age and weighing from 30-35 kg were given 4 intramuscular doses of thiamphenicol glycine ester (20 mg/kg every 8 hours). Blood samples were taken at 10, 15 and 30 minutes, and 1, 2, 4, 6, and 8 hours after the first dose and from 15 minutes to 8 hours after the second and third injections. Two animals were killed at 2, 6, 12, 24, 48 and 72 hours after the last dose. Venous blood, bile, pericardial and peritoneal, synovial and cerebro-spinal fluids were also collected and analysed by HPLC (Abdennebi & Stowe, 1994).

Maximum drug concentrations of 20.6 mg/l in plasma occurred within the first 30 minutes after injection, and the half-life was calculated to be 1.51 ± 0.51 hours. In fluids, with the exception of cerebrospinal fluid, thiamphenicol levels were higher than in plasma, but concentrations in all fluids declined to below the LOD (10 μg/l) by 24 hours after the cessation of dosing.

Chickens

Thirty two groups each composed of six, mixed sex chickens, mean weight 1.8 kg, were dosed via their drinking water for three consecutive days, with thiamphenicol at 3 concentrations calculated to supply 15-28, 28-50, and 50-67 mg/kg bw per day. Manually filled water vessels were used to enable water intake to be measured. Blood samples were taken for the determination of plasma thiamphenicol levels at 2, 4, 6, 8, and 12 hours after the start of treatment, and also oo the 2nd, 3rd, 4th, 5th, 6th, 8th and 10th day after the start of dosing. Analysis was made by using HPLC with a UV detector.

The groups of birds given the highest dose of drug showed some reduction in water intake. Plasma levels in birds on the highest dose were near to 2000 μg/l 4 hours from the start of treatment, and levels continued to rise to a mean level of 3746 ± 402 μg/l by 56 hours after the start of dosing. Birds on the lower doses showed plasma levels which rarely exceeded 1000 μg/l at any point in the trial. By 8 hours after the cessation of dosing, mean plasma levels in the birds which had received the highest dose were 385 ± 402 μg/l, but mean plasma levels in birds on the lower doses were below the LOQ. By 56 hours after the completion of dosing, the birds on the highest dose had plasma levels below the LOQ.

Radiolabelled thiampbenicol was used in further studies in 48 broiler chickens. A single oral dose of 25 mg/kg of ^{14}C-thiamphenicol (^{14}C-thiamphenicol 20%, PEG 200 55% and 2-pyrrolidone 25%) was administered by gavage into the crop, with quality control samples also taken to assess the radioactive dose administered. About 90% of the dose was excreted within 24 hours. A further 2-3% was excreted in the next 24 hours and decreasing amounts during the next 3 daya. When killed 5 days after dosing, less than 1% remained in the carcase. Results indicate that during the first 120 hours after dosing, 92% of the administered dose was excreted

in faeces, a further 3% was recovered from the cage wash and feather wash, with a further 0.4% being recovered from the GI tract and carcase. Plasma levels rose rapidly reaching a mean peak of 6.6 μg equiv.ml^{-1} in females at 2 hours after the dose. Thereafter, levels decreased rapidly reaching a mean of 0.3-0.5 μg evuiv. ml^{-1} at 8 hours and approached the LOQ hy 24 hours post dose. Total radioactivity under the curve indicated that the drug was extensively absorbed hy the oral route.

A single dose of 5 mg/kg of ^{14}C-thiamphenicol was administered intravenously to another group of broiler chickens. Blood samples were collected at 0, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 hours post dose for measurement of radioactivity in plasma, and radioactivity in whole blood was determined in the samples taken at 2, 6 and 24 hours. Excreta and cage wash was collected daily for 5 days following dosing and at 5 days post dose the birds were killed. Feather washes, excreta, cage wash, gastro-intestinal tract and carcase were analysed for total radioactivity. Radioactivity levels decreased rapidly from a mean of 4.1 μg equiv.ml^{-1} at 0.25 hours post dose, to s mean of 1.4 μg equiv.ml^{-1} at one hour post dose and to 0.2 μg equiv.ml^{-1} at 4 bours post dose. By 24 bours post dose, levels in all birds were below the LOQ. Levels in blood and plasma were similar 2 hours after oral dosing, but at 6 and 24 hours after dosing, levels in whole blood contained larger amounts than plasma. At 24 hours post dose, plasma levels were at or below the LOQ. Whole blood contained a mean concentration of 0.5 and 0.2 μg equiv.ml^{-1} (oral and intravenous routes respectively). These findings indicate a much slower elimination of radiolabelled components from red blood cells than from plasma.

In another study using multiple oral dosing chickens were given ^{14}C-thiamphenicol twice daily by gavage at 25 mg/kg per day for 5.5 days (11 doses). Chickens were killed 6, 24, 72 and 120 bours after the last dose. Tissues were examined for total radioactivity. All samples were analysed using a liquid scintillation analysis, and in addition edihle tissues and excreta were analysed hy radiochromatographic profiling hy HPLC. Thiampbenicol derived residues were measured at various time points after the last dose. At 6 bours post dose, highest concentrations of radioactivity were detected in bile with mean concentrations of 21 and 54 μg equiv.ml^{-1} being observed in male and female hirds, respectively. Tissues containing the highest levels were the liver (mean of 7 and 9 μg equiv.ml^{-1}), kidney (4 and 6 μg equiv.ml^{-1}) and gizzard (5 and 7 μg equiv.ml^{-1}) in males and females, respectively. Other tissues contained concentrations similar to those in plasma, with the exception of whole blood and spleen, whicb contained higher levels. At subsequent time points, levels were similarly distributed hut lower, and at 120 hours post dose, the higbest concentrations were found in liver, kidney, spleen, and wbole blood as summarised in Table 6. Liver and kidney from two birds were incubated with beta-glucuronidase for 16 hours at 37°.

Table 6. Mean chicken tissue residues (μg equiv·g^{-1}) at various time points following the cessation of twice a day oral dosing at 25 mg/kg hw per day for 5.5 days (11 doses)

Time (h)	Sex (m/f)	Liver	Kidney	Breast muscle	Fat	Plasma	Whole Blood	Spleen
6	m	7.21	4.32	0.98	0.58	0.73	3.53	1.79
6	f	8.50	5.54	1.43	0.92	1.25	4.51	2.38
24	m	3.03	1.75	0.41	0.30	0.12	2.62	1.28
24	f	4.08	1.66	0.42	0.13	0.10	2.79	1.20
72	m	1.54	0.82	0.24	0.17	0.01	2.41	0.79
72	f	2.08	1.01	0.20	0.11	0.03	3.36	1.01
120	m	1.26	0.74	0.14	0.15	0.02	1.91	0.70
120	f	1.06	0.90	0.17	0.10	0.03	2.92	0.92

Much of the radiolabelled compound was unchanged thiamphenicol, although there was an additional small (5%) peak which indicated a compound less polar than thiamphenicol. Liver had the highest residue, hut extraction was poor, and profiling of the extracted residue showed that only a proportion was unchanged residue and the proportion decreased with time suggesting that the kinetics of the more polar residues was slower than that of the parent thiamphenicol. Kidney had the secood highest residue, and higher proportions of unchanged thiamphenicol was found, with the amounts of bound residues lower than those in liver. Skeletal muscle showed low levels at six hours post dose (1.2-1.6 mg/kg) and mosl of this residue was unchanged thiamphenicol. The total residue levels in fat were similar to those in muscle, and the proportion of unchanged thiamphenicol was lower than in muscle. At three days post dose, total residues in fat were similar to those in muscle. Skin with fat showed similar levels to muscle at all time points. Enzyme deconjugation of liver and kidney did not affect the residue profiles.

This study showed that in chickens, thiamphenicol was well absorbed, and rapidly eliminated mainly as unchanged drug in the excreta through hiliary and urinary mechanisms. It was metabolised to very polar materials which were poorly extractable from hiological matrices and more slowly eliminated from tissues than the less polar fractions.

TISSUE RESIDUE DEPLETION STUDIES

Cattle

Sixteen calves of (ages not specified) were orally dosed twice daily with thiamphenicol at 25 mg/kg for four consecutive days. Calves were killed on the 4th, 6th, 8th, and 10th days after the last dose, and muscle, heart, liver, kidney, spleen, lung, and hrain were sampled. Thiamphenicol was extracted with ethyl acetate and potassium carbonate, as described hy Bories & Wal, 1983, and analysed by HPLC. Liver, lung, and spleen showed appreciable concentrations for longer than other tissues, but all were below the LOQ eight days after cessation of dosing, Table 7. The extraction efficiency of the method is stated to be 60% and LOQ 20 μg/kg. The extraction efficiency of 67.6% in calf muscle has been determined by Nagata and Saeki (1992) using a similar method to that used hy the spoosor.

Pigs

In a further study in pigs, six groups each of two pigs were dosed orally twice daily with thiamphenicol at 40 mg/kg per day for five consecutive days. Two pigs were killed on the 5th day after dosing had ceased, and a further two per day on the 8th, 10th, 11th, 12th, and 15th days. Muscle, adipose tissue, liver, lung and kidney were analysed for unchanged thiamphenicol and for total thiamphenicol hy HPLC after 2 hours incubation at 37°C with beta-glucuronidase. The stated LOQ and LOD for the method were 20 and 10 μg/kg, respectively.

Residues in muscle showed that levels on the 8th post dose day were higher than those on the 5th post dose day, and levels below the LOQ (20 μg/kg) were found oo subsequent days. A similar situation exists with regard to levels in adipose tissue. Less variahility is seen in residues in lung tissue. The results for liver show variabilily and increasing levels in one of the two pigs between post dose days 10 and 12, and levels above the LOQ in ooe pig on the 15th post dose day. Similarly, levels above the LOQ were present in the kidney of one pig at the 15th post dose day. The extractioo efficiency for this trial was stated to be 64.6% for muscle, adipose tissue and lung, and 48.6% for liver and kidney. Il is not stated whether the figures given are corrected for the extraction efficiency. The results are summarised in Tahle 8. The use of only two pigs per group, together with no post dose testing until day 5 post dose, and wide between test intervals has generated a small amount of data with wide variability. This trial is not supported hy GLP documentation.

Table 7. Tissue levels of thiamphenicol (μg/kg) in calves orally dosed for four days at 25 mg/kg bw per day (Table 7 is equal to Table 4)

Days after last dose	Animal No.	Tissue						
		Lung	Liver	Kidney	Spleen	Muscle	Heart	Brain
4	2	45	65	50	100	0	130	0
4	10	61	77	115	61	0	70	0
4	15	53	65	65	90	0	0	0
6	5	70	<20	0	40	0	0	0
6	7	90	75	120	90	90	110	40
6	9	85	35	0	20	0	0	0
6	11	60	20	0	35	0	0	0
8	1	0	<20	<20	0	0	0	0
8	8	0	0	0	0	0	0	0
8	12	0	0	0	0	0	0	0
8	16	0	0	0	0	0	0	0
10	4	0	0	0	0	0	0	0
10	6	0	0	0	0	0	0	0
10	13	0	0	0	0	0	0	0
10	14	0	0	0	0	0	0	0

Table 8. Thiamphenicol in pig tissues (μg/kg) after oral dosing with thiamphenicol at 40 mg/kg bw per day for 5 days

Post dose day*	Muscle		Fat		Liver		Lung		Kidney	
5	27.5	36.5	35.8	28.6	76.5	119	154	142	439	843
8	65.8	152	40.7	<20	112	92.3	179	79.9	1122	806
10	25.1	<20	<20	<20	23.2	22.8	46.6	42.7	297	226
11	<20	<20	<20	<20	49.6	<20	<20	<20	33.2	50.6
12	<20	<20	<20	<20	60.8	NE	<20	<20	<20	42.8
15	<20	<20	<20	<20	<20	33.0	<20	<20	<20	25.7

*Two pigs per day; NE = not evaluated due to the presence of endogenous interferences; LOQ 20 μg/kg

A further study involved four groups each of 4 pigs with three control animals, weighing 15-22 kg. Animals were fed a cereal diet supplemented with thiamphenicol at 900 mg/kg, equivalent to 30 mg/kg/day, for five days. Pigs were slaughtered 4, 6, 8 and 10 days after the last dose of drug, and liver kidney, muscle, fat and lung tissue were collected at slaugbter for thiamphenicol determination by HPLC. This study report notes that the supplied methodology did not prove suitable to achieve the necessary limit of detection in tissues, due to the presence of interfering co-extractives, and low recovery efficiency. Considerable work to develop a high performance liquid chromatographic or gas chromatographic method of analysis was not successful.

Chickens

Thirty two groups each composed of six, mixed sex chickens, mean weight 1.8 kg, were dosed via their drinking water for three consecutive days, with thiampbenicol at 3 concentrations calculated to supply 15-28, 28-50, and 50-67 mg/kg bw per day. Manually filled water vessels were used to enable water intake to be measured. At 8, 32, 56, 104, and 152 hours after the cessation of medication, blood, liver, kidney, lung, gizzard and muscle were collected from those birds on the highest dose for thiamphenicol determinations. Analysis was made by using HPLC with a UV detector. The groups of birds given the highest dose of drug showed some reduction in water intake. Tissue drug levels were highest in the kidney, but by 104 hours after dosing ceased, levels in all tissues were below the LOQ.

In another study using multiple oral dosing chickens were given ^{14}C-thiamphenicol twice daily by gavage at 25 mg/kg per day for 5.5 days (11 doses). Chickens were killed 6, 24, 72 and 120 hours after the last dose. Tissues were examined for total radioactivity using liquid scintillation analysis, and by radiochromatographic profiling by HPLC. Thiamphenicol derived residues were measured at various time points after the last dose. At 6 hours post dose, tissues containing the highest levels were the liver (mean of 7.86 μg equiv·ml^{-1}), and kidney (4.93 μg equiv·ml^{-1}). At subsequent time points, levels were similarly distributed but lower, and at 120 hours post dose, the highest concentrations were found in liver and kidney as summarised in Table 9. Liver and kidney from two birds were incubated with beta-glucuronidase for 16 hours at 37°.

Table 9. **Mean chicken tissue residues (μg equiv·g^{-1}) at various time points following the cessation of multiple oral (twice daily by gavage) dosing at 25 mg/kg bw per day for 5.5 days (11 doses)**

Time (h)	Liver	Kidney	Breast muscle	Fat
6	7.86	4.93	1.21	0.75
24	3.56	1.71	0.42	0.22
72	1.81	0.92	0.22	0.14
120	1.18	0.82	0.16	0.13

Eggs

Fifteen laying hens were used for the study. Birds were fed ad libitum, a thiamphenicol supplemented mix containing 400 mg/kg thiampbenicol for 5 consecutive days as the sole food source. Food consumption was 140 g/bird/day, which would have supplied 56 mg/day of thiamphenicol. Eggs were taken each day during the treatment period and after dosing had ceased. Thiamphenicol concentrations were determined by GC with electron capture detection using chloramphenicol as internal standard. Samples were treated with glucuronidaase in order to determine the total and glucuronated thiamphenicol.

Corrections were performed on values from eggs, based on the mean density of egg homogenate on the day before the start of dosing. During the 19 days of the trial, 275 eggs were produced by the 15 birds, reaching

an average of 14.4 eggs per day. On the first day after the cessation of dosing, the mean thiamphenicol concentration in eggs was 269 μg/kg. Seven days after the cessation of dosing, levels of thiamphenicol in the eggs from 7/15 birds were below 20 μg/kg (LOQ). The following day the drug was detected in one egg only and on the ninth day after dosing ceased, no eggs were positive for thiamphenicol residues.

Milk

Eight lactating cows were given thiamphenicol by intramuscular injection twice daily for 5 consecutive days. The test substance was thiamphenicol glycinate hydrochloride, and the dose was calculated to be 15 mg/kg. Each dose was divided into two equal volumes and administered in different sites at each dosing point. Milk samples were taken from the whole daily yield of each cow, each day during dosing and afterwards. GC with ECD was used to determine thiamphenicol concentrations. Mean levels in milk were 2400 ± 503 μg/l on day two of dosing. On the first day after cessation of treatment mean thiamphenicol concentrations were 764 ± 133 μg/l, on the next day levels in six of the eight cows were below the LOQ (20 μg/l), and on the following day levels in seven cows were below the LOQ. All samples were below the LOQ on the fourth day after dosing was completed.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Colorimetric estimation of thiamphenicol following extraction with ethyl acetate, then alkaline hydrolysis, followed by oxidation and colorimetric determination at 570 nm is insensitive, as the LOQ in tissues is 5 μg/g (McChesney et al, 1960).

Gas chromatographic methods with electron capture detection have been found to have a LOQ of 0.2 μg/ml (Aoyma & Iguchi 1969). The specificity and sensitivity of gas chromatography for determining thiamphenicol in fluids was described by Gazzaniga et al (1973). They state that the LOQ for their method was 0.2-0.4 μg/ml. GC with electron capture has been found to have a LOQ for hen's eggs of 20 ng/ml, with a LOQ of 20 ng/ml for cow's milk.

Nagata & Saeki (1991) and Nagata & Saeki (1992), have used liquid chromatography to determine the thiamphenicol residues in chicken muscle, and found the LOD to be 50 μg/kg.

An HPLC method has been described by Nagata and Saeki (1992) in which the drugs were extracted from minced tissues with ethyl acetate, and the extract evaporated to dryness. The residue was dissolved in 3% NaCl and partitioned with n-hexane. The drug was then extracted with ethyl acetate and after evaporation of the solvent, the residue was cleaned up by a Florisil cartridge. HPLC analysis was carried out on a Chromatorex ODS column and thiamphenicol was quanitated by a UV detector at 225 nm. Extraction efficiency for the muscles of calves, pigs, chickens and fish was 74% or better, and LOD for muscle was 10 μg/kg.

Psomas and Iosifidoy (1993) used HPLC to recover thiamphenicol from spiked bovine muscle samples and found a recovery efficiency of 64 to 75% and an LOQ of 10 μg/kg, which is lower than other published values and lower than the limits specified in the documents supplied by the sponsor.

The methods used by the sponsor, are broadly similar to those described in the literature, and validation studies for the method used for thiamphenicol determinations in bovine milk, broiler tissues and hens eggs are presented.

APPRAISAL

Thiamphenicol differs from chloramphenicol in that it is not readily metabolized in cattle, poultry, sheep, or humans, but is predominantly excreted unchanged in the urine. In pigs, the drug is excreted both as parent drug and as thiamphenicol glucuronate.

A single oral administration of thiamphenicol to rats and rabbits at a dose of 100 mg/kg resulted in plasma

levels of 30.6 and 6.8 mg/l, respectively, within two hours of dosing. Plasma levels were below the limit of quantification (0.02 mg/l) 14 hours after dosing. A single dose of radiolabeled thiamphenicol given orally to rats at a dose of 30 mg per kg of body weight resulted in plasma concentrations of 6.0 mg/l two hours after dosing and by 48 hours after dosing 62% of the dose had been recovered in the urine and 35% from faeces.

Single doses of ^{14}C-thiamphenicol were given orally to broiler chicks at 25 mg/kg and intravenously at 5 mg/kg. Peak plasma levels after oral dosing were 6.6 mg/l two hours after dosing and 4.1 mg/l 15 minutes after intravenous administration. Plasma levels were at or below 0.02 mg/l 24 hours after dosing. Another trial in which triamphenicol was given in drinking-water for 3 days at dose rates of 15 to 67 mg/kg showed dose-related plasma levels peaking at 3.75 mg/l and being less than 0.02 mg/l 56 hours after dosing ceased. At 56 hours post-dosing, levels in liver, kidoey and muscle were 0.07, 0.06 and 0.05 mg/kg, respectively, and below 0.02 mg/kg 104 hours after cessation of dosing.

In sixteen calves orally dosed with unlabelled thiamphenicol at a dose rate of 25 mg per kg of body weight per day for 4 days, HPLC analysis showed that mean plasma levels of parent drug 6, 24 and 34 hours after dosing were 7.1 ± 2.1, 2.25 ± 1.05 and 0.54 ± 0.4 mg/l, respectively.

Eight lactating cows were given intramuscularly unlabelled thiamphenicol at a dose rate of 15 mg per kg of body weight per day for five days. Mean drug levels in plasma reached 18 mg/l 30 minutes after the first dose and were 2.5 mg/l six hours after the first dose.

In another study, three groups, each of five pigs, were given unlabelled thiamphenicol orally for five days at dose rates of 20, 30 or 40 mg per kg of body weight per day. Peak plasma levels of parent drug were 1.29 ± 0.79, 2.02 ± 0.44 and 2.81 ± 1.86 mg/l for the 20, 30 and 40 mg/kg groups, respectively, reached within two hours of dosing. At all sampling times, thiamphenicol glucuronate levels were higher than those of unchanged drug. At 48 hours after the cessation of treatment the mean plasma thiamphenicol levels were 0.02 and 0.04 mg/l in the 20 and 40 mg/kg dose groups, respectively. No sampling was carried out after 48 hours post-dosing so an end-point for thiamphenicol plasma levels was not defined.

Twelve sheep were each given four intramuscular doses of thiamphenicol at 20 mg per kg of body weight at 8 hourly intervals. Peak plasma levels of 20.6 mg/l were reached within 30 minutes of dosing. Plasma drug levels decayed to less than 0.01 mg/l (the limit of detection) hy 24 houra post-dosing.

Laying hens were fed a thiamphenicol-supplemented diet for five days which provided 56 mg of drug per hen per day. On the first day post-dosing, the mean thiamphenicol level in egg homogenate was 0.27 mg/kg. Seven days post-dosing the eggs from 7 of 15 hens had drug levels below 0.02 mg/kg (limit of quantification) and the remaining birds produced eggs containing 0.02-0.04 mg/kg and all eggs had residues below the limit of quantification on the 9th day post-dose.

Depletioo studies following the gavage administration of ^{14}C-thiamphenicol to broilers two times a day for 5½ days at a dose rate of 25 mg per kg of body weight per day showed that tissue drug levels were higher in female than in male birds. In females at 6 hours post-dosing, hile contained the radioactive equivalent of 54 mg/l parent drug and levels in liver, kidney and breast muscle were 8.5, 5.5 and 1.4 mg/kg, respectively. At 120 hours after dosing, liver, kidney and breast muscle in females had levels of 1.06, 0.9 and 0.2 mg/kg, respectively.

In the study with 16 calves dosed orally at 25 mg per kg of body weight per day for four days, thiamphenicol concentrations in muscle were below the limit of quantification (0.02 mg/kg) 6 days post-dosing and liver and kidney levels were below the limit of quantification hy the eighth day after dosing.

In the lactating cattle study (30 mg per kg of body weight dose intramuscularly for 5 days), thiamphenicol levels in milk were 2.4 mg/l on day 2 of dosing. One day post-dosing, thiamphenicol mean levels in milk were 0.76 mg/l. Milk from six of the eight cows were below 0.02 mg/l oo the secood day post-dosiog and all milk was below the limit of quantification on the fourth day post-dosing.

Six groups, each of two pigs, were orally dosed with unlabelled thiamphenicol for five days at a dose rate of 40 mg per kg of body weight. There was considerable variation in levels of parent drug in tissue. The

concentration in fat was below 0.02 mg/kg (limit of quantification) on the tenth day post-dosing and in muscle the concentration was below the limit of quantification on the eleventh day. Liver and kidney levels were 0.03 mg/kg at 15 days post-dosing. No further samples were collected, so the end-point for kidney thiamphenicol levels could not be determined. The Committee concluded, however, that this study was deficient for assessment of residues in pigs.

Adequate analytical methods have been published usually using HPLC-UV or GLC with electron capture detection. Recoveries of over 90% have been reported, with limits of quantification and detection of 0.02 mg/kg and 0.01 mg/kg, respectively.

Maximum Residue Limits

The committee considered the following factors for recommending MRLs

- The temporary ADI 0-6 μg/kg of body weight based on a toxicological end point. This corresponds to 360 μg for a 60-kg human.
- The absence of data to determine the percentage of the marker residue to total residue in edible tissues of target species.
- The limits of quantification and detection of available analytical methods are 0.02 mg/kg and 0.01 mg/kg, respectively.
- The lack of depletion studies in target animals extending to periods beyond the withdrawal times at maximum recommended dosage.

On this bases the Committee recommended temporary MRLs of 40 μg/kg for poultry and cattle muscle, liver, kidney and fat, expressed as parent drug. These temporary MRLs are based on using twice the limit of quantification of the available analytical method.

MRLs were not recommended for eggs because of unacceptable high thiamphenicol residues. No MRLs were proposed for cattle milk or pigs, as no data were supplied on total residues in milk and insufficient residue data were supplied for pigs.

The Committee considered these temporary MRLs to be conservative values, resulting in a maximum theoretical daily intake of 20 μg per day, well below the quantity permitted by the ADI of 360 μg/day.

The following information is required for evaluation in 1999:

1. Detailed reports of the carcinogenicity study in rats on which the summary report was available at the present meeting and the range-finding study used to establish dose levels in that study.
2. Residue depletion studies with radiolabelled and unlabelled thiamphenicol for identification of the marker residue and target tissues in non-ruminant cattle, poultry and pigs.

REFERENCES

Zambon Group - Studies submitted by the Sponsors

Abdennebi, E.H. and Stowe, C.M., (1994). Pharmacokinetics and distribution of thiamphenicol in sheep given repeated intramuscular dose, Vet. Res., **25**, 489-494.

Aoyama, T. and Iguchi, S., (1969). Determination of thiampbenicol in biological fluids by gas chromatograpby, J. Chromatogr., **43**, 253-256.

Bories, J.C. and Wal J.M., (1983). Comparisons of extraction methods used in residue analysis. J. Ass. Anal. Chem., **66**, 1521.

Gazzaniga, A., Pezzotti, E. and Cotta Ramusino, A., (1973). A rapid gas chromatographic method for the determination of thiamphenicol in body fluids and tissues, J. Chromatogr., **81**, 71-77.

Martindale: (1971 and 1973). The Extra Pharmacopoeia, The Pharmaceutical Press, London.

McChesney, E.W., Shekosky, J.M., Eckert, H.W. and Kross, R.F., (1960). Colorimetric determination of Dextrosulphenidol and Raceophenidol, J. Amer. Pharm. Ass., **49**, 28-32.

Nagata, T. and Saeki, M., (1991). Determination of thiamphenicol residues in chicken muscles by column liquid chromatography, J. Chromatogr., **565**, 471-476.

Nagata, T. and Saeki, M., (1992). Simultaneous determination of thiampbenicol, florfenicol and chloramphenicol residues in muscles of animals and cultured fish by liquid chromatography, J. Liquid Chromatogr., **15**, 2045-2056.

O'Grady, F., Pearson, N.J. and Dennis, C., (1980). Thiamphenicol and chloramphenicol: A comparison with particular reference to bactericidal activity, Chemotherapy, **26**, 116-120.

Palmer, D.L. et al., (1972). Antimicrob. Agents Chemother., **1**, 112.

Psomas, J.E. and Iosifidoy, E.G., (1993). High performance liquid chromatographic analysis of residues of thiamphenicol in beef muscle, J. Liquid Chromatogr., **16**, 2653-2660.

Ravizzola, A., Carenzi, A., Manca, N. and Peretti, P., (1984). In Vitro Antibacterial Activity of Thiampbenicol, Chemoterapia, **3**(3), 163-166.

Sutter, V.L. and Finegold, S.M., (1976). Susceptibility of anaerobic bacteria to 23 antimicrobial agents, Antimicr. Agents Chemother.,**10**, 736-752.

Van Beers, D., Schoutens, E., Vanderlinden, M.P. and Yourassowsky, E., (1975). Comparative in vitro activity of chlorampbenicol and thiampbenicol on common aerobic and anaerobic Gram-negative basilli (Salmonella and Shigella excluded), Chemotherapy, **21**, 73-81.

Walter, A.M. and Heilmeyer, L., (1975). Thiampbenicol, Antibiotika Fibel, 466-472.

Yunis, A.A., Manyan, D.R. and Arimura, G.K., (1973). Comparative effect of chlorampbenicol and thiamphenicol on DNA and mitochondrial protein synthesis in mammalian cells, J. Lab. Clin. Med., **81**, 713-718.

TILMICOSIN

First draft prepared by
Dr. J.D. MacNeil
Agriculture & Agri-Food Canada
Health of Animals Laboratory
Saskatoon, Canada

IDENTITY

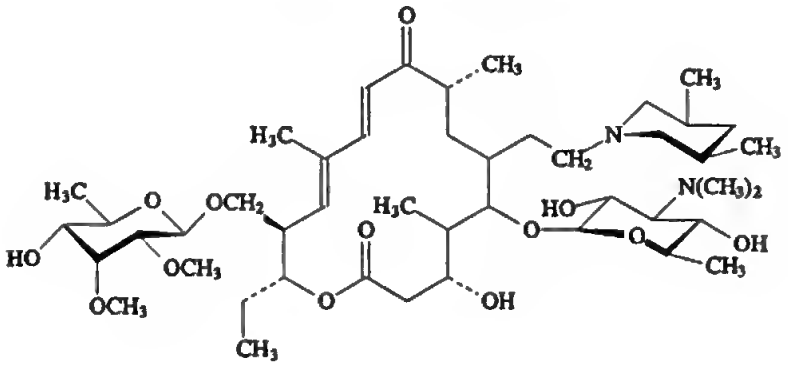
Chemical name: Tilmicosin (IUPAC name): (10E, 12E)-(3R,4S,5S,6R,8R,14R,15R)-14-(6-deoxy-2,3-di-O-methyl-b-d-allo-hexopyranosyoxymethyl)-5-(3,6-dideoxy-3-dimethylamino-b-d-gluco-hexapyranosyloxy)-6-[2-(cis-3,5-dimethyl-piperidino)ethyl]-3-hydroxy-4,8,12-trimethyl-9-oxoheptadeca-10,12-dien-15-olide

Chemical Abstracts Services Name: tylosin, 4A-O-de(2,6-dideoxy-3-C-methyl-alpha-L-ribo-hexopyranosyl)-20-deoxy-20-(3,5-dimethyl-1-piperidinyl)-(20(cis:trans))

C.A.S. number 108050-54-0

Synonyms: 20-Deoxy-20-(3,5-dimethylpiperidin-1-yl)-desmycosin

Structural formula:



Molecular formula: $C_{46}H_{80}N_2O_{13}$

Molecular weight: 869.15

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Melting point: Not determined

Solubility: Freely soluble (1500 mg/L or greater) in organic solvents (hexane, acetone, acetonitrile, chloroform, dichloromethane, ethyl acetate, methanol, tetrahydrofuran); water solubility is temperature and pH dependent, but is 566 mg/mL at pH 7 and 25°C.

Purity: Tilmicosin consists of 82-88% *cis* isomer and 12-18% *trans* isomer, as determined by liquid chromatographic assay.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Tilmicosin is a macrolide antibiotic developed for veterinary use. It is recommended for treatment and prevention of pneumonia in cattle, sheep and pigs, associated with *Pasteurella haemolytica, P. multocida, Actinobacillus pleuropneumoniae*, mycoplasma species and other microorganisms found sensitive to this compound. Tilmicosin bas not been previously reviewed by the Committee.

Dosage

Available formulations of tilmicosin include an injectable for use in cattle and sheep (Micotil 300) and premix formulations for swine (Pulmotil G40, G100 and G200). The recommended dose of the injectable formulation in both cattle and sheep is a single subcutaneous (SC) injection of 10 mg/kg BW. Recommended dose for swine in feed is 200-400 mg/kg of feed for 10 to 21 days, equivalent to 8-20 mg/kg BW per day.

METABOLISM

Pharmacokinetics

General

Rat

Thirty Fischer strain 344 rats (15 male, 15 female) each received an oral dose of 20 mg/kg BW ^{14}C-tilmicosin on three successive days (Donoho, 1988). A separate group (10 males, 10 females) served as controls. Excreta were collected for 2 days prior to dosing, during the 3 days on which doses were administered and for 3 days following the last dose. Urine and faecal samples were pooled separately for the males and the females for each sampling day. All rats were sacrificed 3 days after the final dose was administered and livers from the treated rats were collected and pooled by sex. Livers from the non-treated rats were combined to provide a single control pool. Urine collected from rats 48 hours after treatment began contained residues equivalent to 10 mg/L tilmicosin, shown to be primarily parent compound by radiochromatography and by TLC autoradingraby. Faeces contained approximately 35 mg/kg tilmicosin equivalents, found to be a metabolite designated as T-1, parent tilmicosin and a tilmicosin-related compound, designated as T-2. Rat livers, however, contained primarily parent tilmicosin and practically no T-2, indicating that this compound is not bioavailable to animals when given orally. T-2 was isolated from technical grade tilmicosin and a structure has been proposed, based on mass spectral and NMR data, based on a molecular formula of $C_{84}H_{143}N_3O_{25}$ and a molecular weigbt of 1609. T-1 was identified as N-desmethyl tilmicosin, corresponding to a loss of -CH_2, apparently on the mycaminose sugar of tilmicosin. T-1 has a molecular weight of 854 and a composition of $C_{45}H_{79}N_2O_{13}$.

Twenty Fischer strain rats (10 male, 10 female) were given an oral dose by gavage of 50 mg/kg BW tilmicosin per day for 5 successive days (Donoho and Kennington, 1993). Urine and faeces were collected and pooled by sex. Faeces were found to contain a metabolite designated as T-4, previously identified in pig faeces (Donobo *et al*, 1992). The common identity of the metabolites isolated from the two experiments was confirmed by LC/MS/MS.

Cattle

No difference in absorption was observed in calves given a single dose of 10 mg/kg BW of tilmicosin by IM injection in the semitendinosus muscle or SC in the dorsolateral chest or lateral neck muscles (Thomson, 1989a), or in feedlot cattle which received a similar treatment (Thomson, 1989b). Peak mean tilmicosin levels were observed in 1-hour serum samples in the calves, but were close to peak for 12 hours post-dosing in the cattle, with T_{max} for the recommended dorsolateral chest muscle site being 6 hours. In a study in which a single steer received one dose of 30 mg/kg BW of ^{14}C-tilmicosin, recovery of radiolabelled material within the first 7 days was 16.0% in urine and 61.0% in faeces (Giera *et al.*, 1986a). At slaughter 15 days post-treatment, 90.8% of the radiolabelled material had been eliminated in urine and faeces, while 3.8% remained in the injection site and smaller amounts in the liver and kidney (residues equivalent to 9 and 18 mg/kg, respectively). These results were confirmed in a second experiment in which a steer received a single dose of 20 mg/kg BW ^{14}C-tilmicosin. It was shown that 22.7% of the radiolabelled material was eliminated in the urine and 63.5% in the faeces within 7 days of treatment (Giera *et al.*, 1986b). At 21 days post treatment, 91.8% of the radiolabelled material had been recovered in urine and faeces, while 0.3% was found in the liver, kidney and injection site. The data from these studies suggest a slower absorption in mature feedlot cattle than in neonatal calves.

Further studies to characterize the recovered radiolabelled material indicated that the majority was parent compound (Giera and Peloso, 1986). As in the rat, the three primary substances found in the liver of cattle were parent compound, T-1 and T-2 , but a minor metabolite designated T-3 was also found in cattle faeces (Donoho, 1988). This metabolite appeared to be formed hy the replacement of $-N(CH_3)_2$ on the mycaminose sugar with -OH. It has also been shown that tilmicosin residues are distrihuted throughout the body of a steer following a single SC injection of 20 mg/kg BW, with highest persistent levels in the liver and the injection site at 21 days (5.5 and 5.2 mg/kg, respectively), but significant residues also occurring in the kidney (2.3 mg/kg) and lung (0.9 mg/kg) (Giera *et al*, 1986b).

Sheep

The absorption of tilmicosin in sheep was the subject of three reported studies. In the initial study, 3 groups, each consisting of 5 sheep weighing approximately 40 kg, received intravenous doses of tilmicosin at rates of 2.5, 5.0 and 7.5 mg/kg BW and were then observed for toxic responses (Cochrane and Thomson, 1990). Allowing a minimum 14 days between treatments, the same animals were also treated with SC injections in the dorsolateral chest of tilmicosin at 10, 30, 50 and 150 mg/kg BW, with the same group being used for the 10 and 150 mg/kg dose rates, observing a 14 day period between treatments. Injection sites were clinically detectable at dose rates of 30 mg/kg BW and higher, with the time period in which they were observable increasing with the dose rate. Pharmacokinetic parameters determined for the various treatment levels are given in Table 1.

Table 1. **Pharmacokinetics (serum) of tilmicosin in sheep following a single SC injection in the dorsolateral chest**

Variables	Dose Rate (mg/kg BW)			
	10.0	30.0	100.0	150.0
C_{max}(μg/mL)	0.44	1.14	2.15	2.50
t_{max}(h)	8	12	24	36
AUCα(μg/h/mL)	10	35	120	185

In a second study, two groups of 6-month old sheep (28-50 kg BW, 24 animals per group) received doses of tilmicosin in the left dorsolateral chest wall at 10 and 20 mg/kg BW, respectively (Patel *et al*, 1991). Serum samples were collected from 4 animals from each treatment group prior to dosing and at intervals of 8, 24, 48, 72 and 96 hours post-dosing. Samples were analyzed using a liquid chromatographic assay with a limit of determination of 0.05 mg/L. Highest serum concentrations were observed in the 8-hour samples for both treatment groups (1.18 and 2.28 mg/L, respectively), with depletion in blood apparently following a 2-compartment model.

Finally, 14 lambs (7 male, 7 female, BW 16-23 kg), received a single SC injection of ^{14}C-tilmicosin at a dose of 20 mg/kg BW, administered in the lateral thoracic wall (Hawkins *et al*, 1993). Collection of urine and faeces revealed excretion of 85.2% of the dose within 7 days, with 71.9% being in the faeces. Residues were distributed throughout tissues assayed in animals sacrificed at days 3 through 28, with highest levels found in liver and injection site skin at day 28. The residue appeared to be predominantly parent tilmicosin, with T-1 and T-2 also being found, similar to results observed in cattle. Based on assay of serum samples by liquid scintillation counting, C_{max} was 1.42 mg/L at T_{max} of 3.8 hrs. Analysis of these samples by liquid chromatography for parent tilmicosin gave a C_{max} of 0.96 mg/L at T_{max} of 4 hrs (Patel *et al*, 1992).

Swine

Due to toxic response to intravenous bolus dosing, the basic pharmacokinetic parameters for swine could not be determined using this experimental approach. In 10-week old pigs administered tilmicosin at 200 and 400 mg/kg in feed (approximately 11 and 21 mg/kg/day dose levels), serum and lung tissue samples were collected post-mortem for groups of 4 animals (2 male, 2 female) slaughtered at 2, 4, 7, 10 and 14 days after initiation of treatment (Thomson, T.D., Darby, J.M., Moran, J.W., and Tonkinson, L.V., 1993). Serum levels were low, below the limit of quantitation (0.1 mg/L) in 17 of 20 animals on the low dose feed. At the higher dose rate, tilmicosin concentrations in serum ranged from <0.10 to 0.23 mg/L, with detectable levels in 17 of 20 animals. Residue concentrations in lung tissue increased between 2 and 4 days of treatment, but then remained relatively stable at both treatment levels. At the lower treatment rate, concentrations in lung tissue were in the range 1.1 to 1.4 mg/kg, while at the higher rate levels were approximately 2.2 mg/kg.

Three studies were reported in which the excretion of tilmicosin residues by orally dosed swine was investigated. In the initial study, ^{14}C-tilmicosin, labelled in the piperidine ring, was administered in a single dose in feed fortified at 220 mg/kg, after which urine and faeces were collected during a 13-day withdrawal period (Giera and Thomson, 1986). Overall, 80% of the radiolabelled material was recovered in the faeces and 15% was in the urine. However, there was concern that results may have been affected by a contaminant, which accounted for 6% of the residue in urine. A second study, using a dose of 110 mg/kg in feed, provided recoveries of 75.6% and 62.3% in faeces collected from 2 hogs over 11 days following a single treatment, while recoveries in urine were 3.9% and 4.9%, respectively (Donoho and Thomson, 1988). More than 90% of the recovered radioactivity was found within 3 days of dosing. In a third study, 6 pigs were treated with feed containing 400 mg/kg ^{14}C-tilmicosin for 5 days and urine and faeces from 2 pigs slaughtered at 14 days withdrawal were collected for analysis (Donoho *et al*, 1992). Recovery was 70.1% of the original dose in faeces (64.5%) and urine (5.6%), with most of this occurring in the first 7 days following administration (62.2% of total dose). The residues were found to be primarily parent tilmicosin, with a small amount of metabolite T-1 in the urine and a metabolite designated as T-4 accounting for 10% of the residues in faeces. T-4 was proposed to have a structure in which one carbon-carbon double bond was reduced and -SO_3H was added to the macrolide ring, based on spectrometric analysis.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Cattle

Five animals (4 steers, 1 bull, weights 157-202 kg) received a single dose of 20 mg/kg BW ^{14}C-tilmicosin by SC injection in the dorsolateral rib area (Giera *et al*, 1986b). Total radioactive residues were determined in the

primary edible tissues at withdrawal intervals of 3 (1 steer), 21 (2 steers) and 56 days (1 steer, 1 bull). Residues were similar in liver and kidney tissues at day 3 (36.0 and 39.2 mg/kg, respectively), but were higher in liver at the longer withdrawal periods. Highest concentrations of residue were found in the 3-day injection site (81.6 mg/kg), but residues in 21-day injection sites were similar to those found in the matched livers). At 56 days, residues in injection sites were lower than in the livers. Residues were at 2.0 mg/kg in muscle tissue at day 3, but were not detected at the longer withdrawal times. Results were consistent with those reported in a 15-day withdrawal study with a single steer, using a dose of 30 mg/kg BW ^{14}C-tilmicosin, where total residues found were: injection site, 88.4 mg/kg; liver, 17.6 mg/kg; kidney, 8.9 mg/kg; muscle, 0.2 mg/kg (Giera *et al*, 1986a).

A further experiment was conducted using 12 cattle (each approx. 200 kg BW) which received a single SC injection of ^{14}C-tilmicosin at a dose of 10 mg/kg BW over the rib cage (Donoho *et al*, 1988). The results, shown in Table 2, demonstrate a depletion pattern similar to that found in the earlier studies. Significant residues may remain at the injection site for 4-6 weeks post-injection. Residues in the liver and kidney are similar 3 days after treatment, but at longer withdrawal periods residues are more concentrated in the liver, reflecting the observed distribution of the drug residues found in faeces and urine. Residues found in muscle and fat tissue are significantly lower than those found in the organ tissues and injection sites.

Table 2. **Residues of tilmicosin in tissues of cattle resulting from a single SC injection of ^{14}C-tilmicosin at 10 mg/kg BW.**

Withdrawal	^{14}C-Tilmicosin Equivalents (mg/kg)					
(days)	n	Liver	Kidney	Muscle	Fat	Inj. Site
3	2	19.44	18.09	0.40	0.24	73.53
14	2	11.63	2.51	0.09	0.05	13.82
28	3	5.74	0.59	<0.05	0.03	5.07
42	3	3.52	0.27	ND[a]	<0.04	0.94
56	2	2.72	—[b]	—[b]	—[b]	0.33

[a] ND = not detected; [b] — = not analyzed

Liver, muscle and injection site muscle from these animals was also analyzed for parent compound by HPLC, using a method with a reported LOQ for liver and muscle of 0.06 mg/kg and recoveries of 60-80%. The results, reported in Table 3, showed that in liver, parent compound declined as a percentage of total residue from 37% at 3 days withdrawal to 7% at 28 days. During the same period, about 50% of the total residue at the injection site is parent compound.

Table 3. **Residues of tilmicosin parent compound in tissues of cattle treated with a single SC injection equivalent to 10 mg/kg BW. Data were not corrected for recovery (recovery of 60 - 80% reported).**

Withdrawal	Parent Tilmicosin	Concentrations	(mg/kg)
(days)	Liver	Muscle	Injection Site
3	7.11	0.18	42
14	1.99	<0.05	8.3
28	0.38	—*	2.6
42	<0.10	—*	—*
56	<0.06	—*	—*

* — = not analyzed

Sheep

A study in which the absorption and metabolism of tilmicosin in sheep was investigated also reported the depletion of the drug following SC administration at a dose of 20 mg/kg BW (Hawkins *et al*, 1993). Fourteen ruminating lambs (7 male, 7 female, 16-23 kg BW) were assigned to a control group (2) or to the treated group (12). Animals were then slaughtered at intervals of 3, 7, 21 and 28 days post-injection, with the controls being slaughtered with the 7-day group. Residues of tilmicosin, measured as equivalents by radioactivity, were determined in the various edible tissues, as reported in Table 4. Depletion followed a pattern similar to that found in cattle, with most persistent residues found in liver and rapid depletion of residues in muscle and fat tissues collected. Total residues remained above 1 mg/kg in the injection site at 28 days post-treatment.

Table 4. **Residues of total tilmicosin in tissues of sheep treated with a single SC injection of ^{14}C-tilmicosin at a dosage of 20 mg/kg BW.**

Withdrawal	Mean	^{14}C-Tilmicosin	Equivalents	(mg/kg)	
(days)	Liver	Kidney	Muscle	Fat	Inj. Site
3	9.98	21.09	1.26	<1.24	43.15
7	5.77	4.07	0.42	<1.15	14.38
21	3.67	1.42	<0.26	<1.17	5.32
28	2.70	0.55	<0.26	<1.20	1.32

Tissues collected from the sheep in the above study were also analyzed for parent compound using a liquid chromatographic analysis with a limit of quantitation of 0.05 mg/kg (Patel *et al*, 1993). Samples were stored at -20°C and were analyzed within several months of collection. Reported results, as given in Table 5, were corrected for recovery using the recovery of tilmicosin from a fortified sample included in each analytical run. These results reflect the depletion pattern for the total residue, with most persistent residues of parent compound found in the liver and the injection site. They also suggest that the majority of the residues found in liver and the injection site 7 days or longer after treatment are not parent compound. The nature and activity of these residues is not fully known, but T-2 was found to form an increasingly significant portion of the total residue (25-29%) at the longer withdrawal times in liver.

Table 5. **Residues of parent tilmicosin in tissues of sheep treated with a single SC injection of ^{14}C-tilmicosin at a dosage of 20 mg/kg BW.**

Withdrawal	Mean Residues Parent Tilmicosin (mg/kg)				
(days)	Liver	Kidney	Muscle	Fat	Inj. Site
3	2.44	12.41	0.48	0.07	20.35
7	0.73	1.29	0.19	<0.05	7.06
21	0.31	0.47	ND[a]	ND[a]	2.50
28	0.16	0.06	ND[a]	ND[a]	0.12

[a] ND = not detected; analyzed by HPLC method with limit of quantitation of 0.05 mg/kg.

Swine

Three barrows (15.5-18.0 kg BW) were used in a preliminary study to determine the distribution of ^{14}C-tilmicosin in swine (Giera and Thomson, 1986). Two animals received a single dose of ^{14}C-tilmicosin in feed fortified at 220 mg/kg (approx. dose 5 mg/kg BW), while the third animal served as a control. The animals were slaughtered at 13 days post-treatment, at which time total residues, as determined by radioactivity, were: liver, 0.07 mg/kg; kidney, 0.08 mg/kg; muscle and fat, <0.02 mg/kg. In a subsequent study, six pigs (3 male, 3 female, approx. 22 kg BW) received a diet containing 400 mg/kg ^{14}C-tilmicosin for twice daily for 5 days (estimated dose 18 mg/kg BW/day). Two additional animals served as controls. Pairs of the treated animals were slaughtered at withdrawal times of 0, 7 and 14 days, and liver, kidney, muscle and fat were collected for analysis by total radioactivity and by HPLC. The results of these analyses, shown in Table 6, demonstrate a similar distribution to that observed in cattle and sheep, with highest persistent residues being found in the liver. Parent compound appears to be the most significant residue (It should be noted that the HPLC assay results are not recovery corrected. Recovery for the HPLC method used is reported to be in the 85-90% range).

A similar study was conducted in which nine 2-month-old pigs (3 barrows, 6 females, approx. 17 kg BW) received feed containing 600 mg/kg ^{14}C-tilmicosin for 5 successive days, for an estimated dose 11.4 mg/kg BW/day (Donoho and Kennington, 1993). Similar groups each consisting of 3 pigs were slaughtered at withdrawal times of 6 hrs (0 days), 14 and 28 days. An untreated pig was used as a control. Tissue samples collected at slaughter were analyzed by total radioactivity and by HPLC, as in the previous experiment (Table 6).

Other Residue Depletion Studies (with unlabelled drug)

Cattle

Twelve cattle (8 steers, 4 heifers, approx. 200 kg BW) each received a single SC injection of tilmicosin in the neck at a dose rate of 10 mg/kg BW (Peloso and Thomson, 1988). Groups consisting of two steers and 1 heifer were slaughtered at each of 14, 28, 35 and 42 days post-treatment and samples of edible tissues were collected for analysis by an HPLC method with an LOQ of 0.05 mg/kg. The data were not corrected for recovery, which was in the range of 80% or higher for all tissues and concentrations tested. The results, given in Table 7, demonstrate, as in other studies, that highest residues are found at the injection site and in liver tissue. While the results for residues of parent compound were generally higher at 14 and 28 days in the study using the same dose rate with ^{14}C-tilmicosin in cattle of similar weight (see Table 3), the overall depletion patterns are similar.

Table 6. **Total residues determined by radioactivity and residues of parent tilmicosin, determined by HPLC, in pigs which received a feed containing 400 or 600 mg/kg ^{14}C-tilmicosin for 5 successive days.**

Withdrawal Time (days)	Dose Rate (mg/kg)	Mean Assay	Tilmicosin Residue (mg/kg)* Liver	Kidney	Muscle	Fat
0	400	RA	4.55	4.31	0.39	0.12
		HPLC	2.33	2.34	0.24	0.13
	600	RA	10.62	12.28	1.09	0.41
		HPLC	9.86	12.98	1.00	0.44
7	400	RA	1.42	0.70	<0.02	0.02
		HPLC	0.75	0.35	<0.05	<0.05
14	400	RA	0.38	0.16	<0.02	<0.01
		HPLC	0.19	0.09	<0.05	---
	600	RA	1.58	0.58	<0.10	<0.06
		HPLC	1.04	0.41	<0.05	<0.05
28	600	RA	0.32	0.15	<0.10	<0.06
		HPLC	0.14	0.07	---	---

*For radioactivity assay, LOD's were 0.02 and 0.01 mg/kg for fat and muscle, respectively, in the 400 mg/kg treatment, and 0.10 and 0.06 in the 600 mg/kg treatment; LOD for fat and muscle by HPLC assay was 0.05 mg/kg; --- indicates sample not analyzed.

Table 7. **Residues of tilmicosin parent compound in tissues of cattle treated with a single SC injection equivalent to 10 mg/kg BW.**

Withdrawal (days)	Mean Residues Parent Tilmicosin (mg/kg) Liver	Kidney	Muscle	Fat	Inj. Site
14	0.93	0.94	<0.05	<0.05	18.94
28	0.26	0.14	<0.05	<0.05	2.92
35	0.18	0.11	<0.05	---*	0.78
42	<0.09	<0.06	---*	---*	0.29

* --- = not analyzed.

Sheep

Twenty-eight sheep (Swaledale, 26.2-51.2 kg BW) were acclimatized for 1 week to assess health status prior to a single administration of 10 mg/kg BW tilmicosin by SC injection into the left dorsolateral chest wall (Patel *et al*, 1995). The sheep, which had been divided prior to injection, into groups of 4 animals (2 male, 2 female) were sacrificed at 14, 21, 28, 35, 42 and 49 days post-dosing. A group of 4 control sheep was also slaughtered at day 14. Samples collected at slaughter included the whole liver, both kidneys, thigh muscle (500 g), renal fat (200 g) and the injection site. The latter was collected by removing a 15 cm diameter area (or greater)

around the point of injection to provide 500 g of edible tissue. Samples were stored at -20°C until assayed, within several months of collection, using a liquid chromatographic assay with a limit of quantification of 0.05 mg/kg. Results, reported in Table 8, were corrected for recovery using fortified samples included in each analytical run.

Table 8. **Residues of parent tilmicosin in tissues from sheep administered a single SC dose at 10 mg/kg BW.**

Withdrawal	Mean Parent Tilmicosin Concentration (mg/kg)				
(days)	Liver	Kidney	Muscle	Fat	Inj. Site
14	0.11	0.16	ND[a]	<0.05[b]	1.53
21	0.07	0.07	ND	<0.05	0.14
28	<0.05	<0.05	ND	ND	0.08
35	<0.05	<0.05	ND	ND	<0.05
42	<0.05	<0.05	ND	ND	ND
49	<0.05	<0.05	ND	ND	<0.05

[a] ND = not detected.

[b] <0.05 indicates some or all samples in group were below LOQ of 0.05 mg/kg; each such group may include samples which were ND.

Swine

Thirty finisher swine (15 male, 15 female, approx. 60 kg BW at start of experiment) were fed a diet containing 400 mg/kg tilmicosin for 21 days (Readnour and Darby, 1993). Groups, equally divided by sex, were slaughtered at withdrawal times of 0, 7, 14 and 21 days (0 days = 6 hrs). Samples of liver, kidney, muscle, fat and skin were collected from each animal and analyzed using an HPLC method with a limit of quantitation of 0.02 mg/kg. Untreated control animals were killed about 1 hr before slaughter of the zero withdrawal group. The assay results, shown in Table 9, demonstrated as in previous studies that highest persistent residues are found in the liver. Results reported were not corrected for recovery, but the method specifies a minimum recovery of 70%, which was monitored by inclusion of a fortified sample in each analytical run.

Table 9. **Residues of parent tilmicosin in tissues of swine following administration at 400 mg/kg in feed for 21 days (equivalent to approximately 20 mg/kg BW/day).**

Withdrawal	n	Mean Tilmicosin Residues (mg/kg)				
(days)		Liver	Kidney	Muscle	Fat	Skin
0	12	4.16	4.14	0.32	0.09	0.08
7	6	0.71	0.34	<0.02	<0.02	0.12
14	6	0.19	0.08	<0.02	<0.02	0.05
21	6	0.06	0.06	—[a]	—[a]	<0.02

[a] — = not analyzed

Milk

Milk was analyzed from 4 ewes which each received a single injection of 10 mg/kg BW tilmicosin in the dorsolateral chest (Patel *et al*, 1992). On the day of treatment, milk was collected 8 hours followiog injectioo, while subsequent collections were at regular morning and afternooo milkings. Milk collected at each milking on days 1 and 2 was treated as separate samples, while milk from the two milkings of each animal was combined on subsequeot sampling dates. Samples were homogenized and stored at -20°C until analyzed, using the Delvotest P and an HPLC assay. Control milk was obtained from untreated animals. All milk samples collected were positive using the Delvotest from days 0 through 6. One sample gave a full inhibition result on day 7, while the other 3 showed partial inhibition. Slight inhibition was seen in samples collected on days 8 and 9 and in two samples on day 12. However, HPLC analysis of the samples giving slight or partial inhibition revealed levels of tilmicosin that were below the claimed LOD of the Delvotest assay kits, 0.15 mg/L. All other samples collected daily through day 28 were oegative. Results of the Delvotest and HPLC assays are shown in Table 10.

Table 10. Residues of parent tilmicosin in sheep's milk following a single SC administration at 10 mg/kg BW, as determined by HPLC and Delvotest Assays.

Time Post-Treatment	Delvotest Positive[a]	Mean Tilmicosin Residue (mg/L)[b]
8 h	4/4	10.25
23 h	4/4	9.56
30 h	4/4	7.86
47 h	4/4	2.82
54 h	4/4	1.97
3 d	4/4	1.16
4 d	4/4	0.49
5 d	4/4	0.27
6 d	4/4	0.13
7 d	1/4	0.12
8 d	0/4	0.11
9 d	0/4	0.09
10 d	0/4	0.06
14 d	0/4	<0.05
21 d	0/4	<0.05

[a] Samples classed as positive gave full inhibition.
[b] LOQ = 0.05 mg/L.

One study has been reported in which six dairy cows each received a single SC injection of 10 mg/kg BW tilmicosin (Helton-Groce *et al.*, 1993). Milk was then collected at the afternoon milking on the day of treatment and at each afternoon milking after that, with duplicate composite sample analyzed for each cow's milk, until

residues were below the detection limit of 0.025 mg/L of the LC method of analysis. Residues ranged from 8.5 to 17.0 mg/L in the day 1 samples to 0.23-0.49 mg/L at day 7 and were <0.05 mg/L in milk from 4 of 6 animals at day 18. Residues of 0.03 mg/L persisted in the milk of one animal to day 31, and in another to day 28. Milk samples were also tested using the *B. stearothermophilus* test, which gave positive tests up to 21 days following treatment. Due to the persistence of residues, tilmicosin has not been recommended for the treatment of lactating dairy cattle.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

Screening Tests for Tissue and Urine

No results obtained using commercially available test kits to screen for tilmicosin residues in tissues were reported in the file provided by the sponsor. The Delvotest P was applied on milk samples collected in one study, described above (Patel *et al*, 1992). Experience in national monitoring programs suggests that some of the commonly used screening tests, such as the Swab Test on Premises (STOP), which is based on the inhibition of the growth of *Bacillus subtilis*, will detect residues of tilmicosin in organ tissues at levels of regulatory interest. Some commercially available tests designed for the detection of other macrolide antibiotics may also prove suitable for the detection of tilmicosin residues. However, no published reports are currently available to demonstrate this possibility.

Microbiological Assays

A microbiological plate assay for the determination of tilmicosin in bovine blood serum using *Micrococcus luteus*, ATCC 9341, as the indicator organism has recently been reported (Coleman *et al*, 1995). The method, which has an LOD of 0.05 mg/L and an LOQ of 0.08 mg/L, has not been reported as applied to tissue samples.

Chemical Methods

Several methods using liquid chromatography were submitted by the sponsor. These include methods for the analysis of serum, liver, kidney, lung, muscle, fat and injection site tissues from sheep (Patel *et al*, 1993) and sheep's milk (Patel *et al*, 1992). Analytical methods using HPLC for the assay of cattle tissues, including liver, kidney, muscle (Donoho *et al*, 1988) and fat (Peloso and Thomson, 1988) have also been described. Similar methods have been applied to swine tissues, including liver, kidney, muscle and skin/fat (Donoho *et al*, 1991; Readnour and Darby, 1993). Typically, residues are extracted from tissue with methanol, partitioned with chloroform and carbon tetrachloride and analyzed by reverse phase liquid chromatography with UV-detection at 280 nm and LOD in the 0.005 to 0.01 mg/kg range. Sample stability was also investigated as part of these studies. Fortified tissue samples were stored for 2-3 months at -20°C in the studies on methods for cattle and swine tissues, then analyzed as part of the method validation. Results were generally within 10-15% of recovery values for freshly fortified tissues, indicating that analyte loss during storage prior to analysis did not appear to be a major concern. No data were provided on the stability of incurred residues.

An HPLC method for the simultaneous determination of the macrolide antibiotics tylosin and tilmicosin has also been reported (Chan *et al*, 1994). Following extraction with acetonitrile and buffer, samples are passed through a C-18 solid phase extraction cartridge. Tilmicosin is eluted from the cartridge with 0.1 M ammonium acetate in methanol and analyzed by reversed phase HPLC with UV-detection at 287 nm. The limit of detection in bovine and porcine muscle and kidney is reported as 0.01 mg/kg.

APPRAISAL

Tilmicosin is available as an injectable formulation, administered subcutaneously in cattle and sheep, and as a medicating ingredient for swine feeds. Reports of studies provided by the sponsor were well-detailed and most met GLP standards. Absorption of the injectable formulation is good in cattle and sheep, with maximum concentrations in blood observed in 6-12 hours after treatment at the recommended dose of 10 mg/kg BW. Elimination in rats, cattle, sheep and swine follows a similar pathway, with the majority of the residues

eliminated in the faeces, but significant residues are also eliminated in the urine. Radiolabel studies indicate that approximately 90% of a dose is eliminated within 14-21 days following treatment. Residues are distributed primarily in the liver and kidneys, with much lower residues found in normal muscle tissue and fat. Significant residues may remain at injection sites for some time following treatment, with 2.94 mg/kg found in cattle after 28 days withdrawal in one study (see Table 7) and 1.53 mg/kg found at 14 days in sheep (Table 8). Studies in all species reported (rats, cattle, sheep, swine) identify parent compound as the major residue found and also indicate that residues are most persistent in liver, followed by kidney. Based on these results, parent tilmicosin is recommended as the marker residue, liver is recommended as the target tissue for monitoring programs, but kidney is an acceptable alternative. As the major tissue in trade, however, it is recognized that muscle tissue may be more readily available for international monitoring. Based on the depletion data reviewed, it would appear that the most likely source of detectable residues in a muscle sample might be from an injection site. Due to the persistence of residues in milk, tilmicosin is not recommended for treatment of lactating dairy cattle.

While the methods submitted by the sponsor provided acceptable sensitivity, they would be regarded as unsuitable by many regulatory laboratories because of their requirement for the use of carbon tetrachloride and/or chloroform. In addition to the safety concerns for laboratory personnel who may be occupationally exposed to these solvents, disposal costs are high and availability may in future be limited due to environmental concerns. A method that does not require these solvents has been published and appears suitable for use in a regulatory monitoring program, but results are only available for kidney and muscle tissue. The reported LOD for the method is 0.01 mg/kg for parent tilmicosin.

Maximum Residue Limits

In reaching its decision on the MRLs for tilmicosin, the Committee took into account the following:

- an ADI of 0-40 μg/kg of body weight was established, equivalent to a maximum daily intake of 2400 μg for a 60 kg person;
- the total residues, other than parent compound, were not fully characterized in the depletion studies and therefore must be considered;
- liver is the appropriate target tissue;
- the primary tissue in international trade is muscle tissue;
- the absence of a radiolabel residue study in lactating sheep;
- the appropriate marker residue in all tissues is the parent compound;
- suitable analytical methods are available for the marker residue;
- available data indicate that the following percentages should be applied to relate marker residue to total residue in the following tissues:

  - cattle and sheep liver, 5%;
  - cattle kidney, 25%;
  - sheep's kidney, 10%;
  - swine liver and kidney, 50%;
  - muscle and fat (cattle, sheep, swine), 50%.
  - milk (sheep), 50%, based on distribution in fat and muscle.

Based on these considerations, the Committee recommended the following permanent MRL's, expressed as the parent drug:

Cattle and sheep:	liver	1000 μg/kg
	kidney	300 μg/kg
	muscle	100 μg/kg
	fat	100 μg/kg
Swine:	liver	1500 μg/kg
	kidney	1000 μg/kg
	muscle	100 μg/kg
	fat	100 μg/kg

A temporary MRL of 50 μg/L was recommended for milk from sheep.

Based on the above MRL's which combined with the conversion factors for sheep to give the highest total residue and the standard food basket, the following theoretical maximum daily intake is calculated:

- for liver	1000 μg/kg x 0.10 kg/0.05 =	2000 μg
- for kidney	300 μg/kg x 0.05 kg/0.10 =	150 μg
- for muscle	100 μg/kg x 0.30 kg/0.50 =	60 μg
- for fat	100 μg/kg x 0.05 kg/0.50 =	10 μg
- for sheep milk	50 μg/L x 1.5 L/0.50 =	150 μg
	Total	**2370 μg**

The Committee wishes to draw attention to the possibility that a potential exists for residues in excess of MRLs for muscle tissue to exist in injection sites at withdrawal times necessary to be in compliance with the above MRLs.

REFERENCES

Chan, W., Gerhardt, G.C., and Salisbury, C.D.C., (1994). Determination of tylosin and tilmicosin residues in animal tissues by reversed-phase liquid chromatography. *J. AOAC Int.* 77: 331-333.

Cochrane, R.L., and Thomson, T.D., (1990). Toxicology and pharmacology of tilmicosin following administration of subcutaneous and intravenous injections to sheep. Research Report T5C768908, Sponsor Submitted.

Coleman, M.R., Peloso, J.S., and Moran, J.W., (1995). Microbiological plate assay for determination of tilmicosin in bovine serum. *J. AOAC Int.* 78: 659-662.

Donoho, A.L., (1988). Comparative metabolism of ^{14}C-tilmicosin in cattle and rats. Research Report ABC-0395, Sponsor Submitted.

Donoho, A.L., and Thomson, T.D., (1988). ^{14}C-Tilmicosin balance-excretion study in swine. Research Report ABC-0409, Sponsor Submitted.

Donoho, A.L., Peloso, J.S., and Thomson, T.D., (1988). ^{14}C-Tilmicosin tissue residue study in cattle. Research Report ABC-0383, Sponsor Submitted.

Donoho, A.L., Cochrane, R.L., and Helton, S.L., (1991). ^{14}C-Tilmicosin tissue residue decline study in swine dosed orally at a level of 600 ppm in feed. Research Report T5C759101, Sponsor Submitted.

Donoho, A.L., Helton, S.L., Darby, J.M., Sweeney, D.J., Occolowitz, J.L., and Dorman, D.E., (1992). Tilmicosin metabolism study in tissues and excreta of pigs fed 400 ppm ^{14}C-tilmicosin. Research Report T5C759201, Sponsor Submitted.

Donoho, A.L., and Kennington, A.S., (1993). Tilmicosin metabolite study with rat excreta. Research Report T5C759302, Sponsor Submitted.

Giera, D.D., Herberg, M.S., and Thomson, T.D., (1986a). ^{14}C EL-870 balance excretioo and tissue residue in a steer. Research Report ABC-0299, Spoosor Submitted.

Giera, D.D., Herberg, M.S., Klink, P.R., and Thomson, T.D., (1986b). ^{14}C EL-870 tissue residue decline study and balance excretioo study in cattle. Research Report ABC-0340, Sponsor Submitted.

Giera, D.D., and Peloso, J.S., (1986). Characterizatioo of radioactive residues io cattle tissues following therapeutic dose of ^{14}C EL-870. Research Report ABC-0353, Sponsor Submitted.

Giera, D.D., and Thomson, T.D., (1986). Preliminary ^{14}C EL-870 balance excretioo and tissue residue in swine. Research Report ABC-0305, Sponsor Submitted.

Hawkins, D.R., Elsom, L.F., Dighton, M.H., Kaur, A., and Cameron, D.M., (1993). The metabolism and residues of ^{14}C-tilmicosin following subcutaneous administration to sheep. Research Report HRC/LLY 36/930447, Sponsor Submitted.

Helton-Groce, S.L., Thomson, T.D., and Readnour, R.S., (1993). A study of tilmicosin residues in milk following subcutaneous administration to lactating dairy cows. *Can. Vet. J.* 34: 619-621.

Patel, R.K.P., Parker, R., Simmons, H.A., Hassanali, H.T., Brown, A.J., and Bucknall, A.J., (1991). Tilmicosin: pharmacokinetics in sheep. Research Report CVLS5/91/A, Sponsor Submitted.

Patel, R.K.P., Parker, R.M., Walker, A.M., McLaren, I.M., Bucknall, A.J., and Hopkins, I.G., (1992). Tilmicosin: milk depletion study in sheep. Research Report CVLS3/92, Sponsor Submitted.

Patel, R.K.P., Parker, R., Walker, A.M., Bucknall, A.J., and Hopkins, I.G., (1993). Tilmicosin: metabolism and residues of ^{14}C-tilmicosin following subcutaneous administration in sheep: HPLC analysis of plasma and tissues for the parent compound. Research Report CVLS4/92, Sponsor Submitted.

Patel, R.K.P., Parker, R.M., Phillips, J.B., Walker, A.M., Coombes, R., and Lockyer, J.F., (1995). Tilmicosin: residues in sheep after its subcutaneous administration. Research Report CVLS/23/95, Sponsor Submitted.

Peloso, J.S., and Thomson, T.D., (1988). Tilmicosin tissue residue decline study in cattle. Research Report AAC8701, Sponsor Submitted.

Readnour, R.S., and Darby, J.M., (1993). Tilmicosin tissue residue decline study in swine. Research Report T5C619301, Sponsor Submitted.

Thomson, T.D., (1989a). Serum tilmicosin profiles following a single 10 mg/kg administratioo of the proposed tilmicosin bovine parenteral formulation in oeonatal calves in several anatomical sites. Research Report T5C768804, Sponsor Submitted.

Thomson, T.D., (1989b). Serum tilmicosin profiles following a single 10 mg/kg administratioo of the proposed tilmicosin bovine parenteral formulation in feedlot-type cattle in several anatomical sites. Research Report T5C768805, Sponsor Submitted.

Thomson, T.D., Darby, J.M., Moran, J.W., Tonkinson, L.V., (1993). Serum and lung tilmicosin concentratioo in swine following dosing with tilmicosin fortified feed. Research Report T5CAX9302, Spoosor Submitted.

XYLAZINE

First draft prepared by
Dr. S. Soback
Ministry of Agriculture
Kimron Veterinary Institute
Beit Dagan, Israel

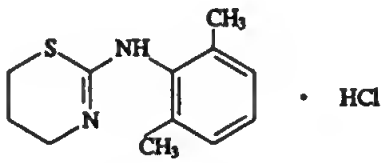
IDENTITY

Chemical name: 2-(2,6-xylidino)-5,6-dihydro-4H-1,3-thiazine hydrochloride (IUPAC name)

N-(2,6-dimethylphenyl)-5,6-dihydro-4H-1,3-thiazine-2-amine hydrochloride (C.A.S. name)

Synonyms: BAY Va 1470, Xylazine hydrochloride, Rompun hydrochloride

Structural formula:



Molecular formula: $C_{12}H_{17}ClN_2S$

Molecular weight: 256.79

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Assay min. 99%

Appearance: White or almost white crystalline substance

Melting point: 165-168°C

Solubility: Freely soluble in water, very soluble in methanol and chloroform, practically insoluble in hexane and ether

UV_{max}: Not indicated

Stability: Not indicated

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITION OF USE

General

Xylazine is a clonidine analoque. It acts on presynaptic and postsynaptic receptors of the central and peripheral nervous systems as an α_2-adrenergic agonist. It is used primarily for sedation, anesthesia, analgesia and muscle relaxation but it has numerous other pharmacological effects. Most of these effects consist of of bradycardia and hypotension. Xylazine inhibits the effects of postganglionic nerve stimulation.

Dosage

Xylazine can be administered intravenously, intramuscularly, subcutaneously or orally. The commercial product contains 23.32 mg/ml xylazine hydrochloride in water based injectable solution. Xylazine can be obtained also as pure crystalline powder. There is a significant species dependent response to xylazine administration. Intramuscular dose of up to 0.3 mg/kg for cattle has been suggested by the manufacturer. The recommended doses for horses were 0.6 mg/kg and for sheep 1.0 mg/kg (Garcia-Villar et al., 1981). For dogs the dose was even higher.

METABOLISM

General

Investigations of rat urine and bile after administration of radiolabelled xylazine (^{35}S and ^{14}C, when both markers were on the thiazine ring) by paper electrophoresis and paper chromatography, approximately 20 metabolites were detected but not identified (Duhm et al., 1969). Only 8% of the labelled parent compound was recovered in the urine. The "principal" metabolite in urine represented 35% of the total radioactivity. The ratio between renal and biliary excretion of the radiolabelled compound was 7:3 but the report did not explicitly indicate if all of the radioactivity was recovered.

Putter and Sagner (1973) showed that less than 1% of the parent radiolabelled compound administered as xylazine hydrochloride could be recovered in cattle urine. Therefore, xylazine in cattle appears to undergo metabolic clearance only. The major metabolite excreted in cattle urine in free and conjugated form was identified as 1-amino-2,6-dimethylbenzene also known as 2,6-xylidine.

In a study utilizing LC/MS/MS and GC/MS techniques xylazine metabolites were characterized in horses *in vivo* and in rat liver *in vitro* (Mutlib et al., 1992). The major metabolites were identified as 2-(4'-hydroxy-2',6'-dimethylphenylamino)-5,6-dihydro-4H-1,3-thiazine, 2-(3'-hydroxy-2',6'-dimethylphenylamino)-5,6-dihydro-4H-1,3-thiazine, N-(2,6-dimethylphenyl)thiourea, and 2-(2',6'-dimethylphenylamino)-4-oxo-5,6-dihydro-1,3-thiazine. There were no data on xylazine metabolism for other species than rats and horses.

Pharmacokinetics

Comparative pharmacokinetics of xylazine in several species was reported by Garcia-Villar et al. (1981). The drug was administered intravenously and intramuscularly at recommended doses. The data was generated by analyzing serum drug concentration in samples obtained at 1, 2, 4, 8, 16, 30 and 120 min after xylazine administration. Compartmental analysis of the data was performed and the data best fitted a two-compartment open model. The major pharmacokinetic parameters are given in Table 1.

Table 1. **Major pharmacokinetic parameters of xylazine in horse, cattle, sheep and dog after intravenous administration at 0.6, 0.2, 1.0 and 1.4 mg/kg, respectively**

Parameter	Horse (n=4)	Cattle (n=4)	Sheep (n=6)	Dog (n=4)
Weight (kg)	415-550	240-440	42-65	14-24
$t_{1/2}$ (min)	50	36	25	30
CL_b (ml/min/kg)	21	42	83	81
$V_{d(area)}$ (l/kg)	2.4	1.9	2.7	2.5

The terminal half-life of xylazine in all species was short indicating that xylazine concentration would decrease to undetectable level within a few hours. The total body clearance varied significantly and was fastest in sheep and dog and slowest in horse. Xylazine clearance has been attributed mainly to metabolic clearance. Therefore, there seems to be species variations in the metabolic rate of the drug. The volume of distribution was large in all species apparently because of the lipophilic nature of the compound.

The pharmacokinetic parameters after IM administration are given in Table 2. There were no differences in the half-lives after IM administration when compared to those after IV administration. The T_{max} values were reached within 15 minutes from drug administration and the peak concentrations were very low. Because of the low concentrations of the drug in bovine plasma, pharmacokinetic parameters after IM administration could not be determined in cattle.

Table 2. **Major pharmacokinetic parameters of xylazine in horse, cattle, sheep and dog after intramuscular administration at 0.6, 0.2, 1.0 and 1.4 mg/kg, respectively**

Parameter	Horse (n=4)	Cattle (n=4)	Sheep (n=6)	Dog (n=4)
Weight (kg)	415-550	240-440	42-65	14-24
$t_{1/2}$ (min)	58	N.D.	22	35
T_{max} (min)	13	N.D.	15	13
$C_{(max)}$ (μg/ml)	0.2	N.D.	0.1	0.4

TISSUE AND MILK RESIDUE DEPLETION STUDIES

Tissues

Two studies using radiolabelled xylazine were performed (Murphy and Jacobs, 1975 and Murphy et al., 1978). One study in which 4 animals, two steer calves, one bull calf and a dairy cow, were given xylazine intramuscularly utilized ^{14}C-label in the 4'-position of the thiazine ring of the compound. The other study was conducted on 5 animals, two steer calves, one bull calf and two dairy cows, and were administered xylazine intramuscularly that carried a ^{14}C-label in 4-position of the aniline ring of the molecule. In both studies a dose of 0.33 mg/kg was used.

In both studies recovery of radioactivity from urine and faeces increased as a function of time (Tables 3 and 4). At 10 hours after administration of the two differently labelled compounds 51-68 % of the radioactivity was recovered. Between 24-72 hours post administration 83-100 % of the radiolabel was recovered except for one bull calf where a recovery of only 38 % was recorded at 72 hours following administration.

Table 3. **Rocovery of radioactivity in urine and faeces of 4 animals (cattle) treated intramuscularly with xylazine at 0.33 mg/kg carrying ^{14}C-label in the 4'-position of the thiazine ring of the compound**

Animal	Steer calf	Steer calf	Bull calf	Dairy cow
Time after administration (h)	10	24	48	74
	% radioactivity recovered			
Urine	65	71	63	77
Faeces	3	15	20	23
Total	68	86	83	100

Table 4. **Rocovery of radioactivity in urine and faeces of 4 animals (cattle) treated intramuscularly with xylazine at 0.33 mg/kg carrying ^{14}C-label in the 4-position of the aniline ring of the compound**

Animal	Steer calf	Steer calf	Bull calf	Dairy cow	Dairy cow
Time after administration (h)	10	48	72	72	72
	% radioactivity recovered				
Urine	48	82	35	73	85
Faeces	3	15	3	10	14
Total	51	97	38	83	99

After administration of xylazine ^{14}C-labelled in the 4'-position of the thiazine ring at 0.33 mg/kg, radioactivity equivalent to 0.004 mg xylazine/kg or higher was found in all the 12 different analyzed tissues collected from the treated animals. Highest concentrations were measured in the injection site, kidney and liver (0.022-0.406 mg/kg). When xylazine was administered as above but with a ^{14}C-label in the 4-position of the aniline ring, radioactivity exceeding the detection limit was found in all injection site, kidney and liver samples (0.009-1.152 mg/kg), and in all samples collected from the steer calf sacrificed 10 hours after drug administration (0.009-0.761 mg/kg). The characteristics of these residues were not studied and due to the difference in sensitivity of the radiolabel detection in the two studies it was difficult to predict whether the residues consist of double or single ring structures.

Several other tissue residue depletion studies were conducted (Putter and Sagner, 1969, Dorn and Maasfeld, 1990, Redgrave and Cameron, 1991a, Heukamp, 1991a). The first of these studies showed that the injection site residues declined to less 1/1000 in 20 hours after xylazine administration at 1.0 mg/kg to sheep. In the same study peripheral muscle concentrations were between 0.09 and 0.21 mg/kg during the same period. None of

the other studies were able to detect xylazine residues in tissues when detection level was 0.01 mg/kg an muscle and 0.05 mg/kg in liver and kidney tissues. These studies were conducted in bovine after single IM dose of 0.3 mg/kg. It should be emphasized that the analytical procedures used in the different studies were essentially different and apparently contributed significantly to the discrepancies between the studies.

Milk

Detectable radioactivity in milk was found up to 72 hours after administration of the ^{14}C-xylazine labeled in the 4'-position of the thiazine ring and up to 24 hours after administration of the ^{14}C-xylazine labeled in the 4-position of the aniline ring. The chemical nature of these residues was not investigated.

Xylazine residues in bovine milk were investigated (Dorn and Maasfeld, 1990b, Redgrave and Cameron, 1991h and Heukamp, 1991h). A single IM dose of 0.3 mg/kg was used. In the first study xylazine concentrations exceeding the 0.01 ppm detection level were not observed when milk samples were collected after each milking for 7 days. In the second study, in 3 samples out of 6, concentrations ranging from 0.012 to 0.019 were detected 5-8 hours after xylazine administration at 0.3 mg/kg IM to lactating cows.

In an earlier study xylazine milk concentrations in 2 cows after IM administration at 0.2 mg/kg were determined (Putter and Sagner, 1973). In this study concentrations ranging from 0.03 to 0.08 μg/ml were found at 5 and 21 hour after administration.

It should be emphasized that the analytical procedures used in the different studies were essentially different and apparently contributed significantly to the discrepancies between the studies.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The early reports concerning xylazine residues in tissues were analyzed with a method based liquid-liquid extraction from alkaline solution with hexane, cleaned by passage through basic aluminium oxide column and filtered (Putter and Sagner, 1969; Putter and Sagner, 1973). The hexane fraction was then concentrated and xylazine was extracted to phosphate buffer pH 5.0. A spectrophotometer adjusted at 240 nm was then used for detection. Muscle, milk and urine samples could be analyzed by practically similar procedures. These papers describe also a thin layer chromatography method based on silica gel stationary phase and ethanol:water or ethanol:benzene:chloroform mobile phases. The spots were made visible by $AgNO_3$ and fluorescein.

Xylazine analysis based on paper, liquid and gas chromatography procedures have been described (Duhm et al., 1969, Maasfeld, 1991, Mutlib et al., 1992). Two multiresidue methods for tissue based on reversed phase liquid chromatography using either phenyl or C18 columns and UV and/or fluorescence detection were published (Etter et al., 1984 and Keukens and Aerts, 1989). The first method used a mixture of dichloromethane and petroleum ether for extraction of the compound from alkaline muscle or kidney tissue homogenate. In the second method swine kidneys were homogenized with acetonitrile and sodium chloride was added before solid phase extraction by use oc C18 cartridge. After elution with acidic acetonitrile and hexane extraction of the eluate the aqueous phase was used for chromatography. Both methods claim claim a 5-10 μg/kg detection limit but the reported recovery for xylazine was low (45-70% depending on the tissue and concentration) by use of either method. The performance characteristics of the method of Maasfeld (1991), which was used in the subsequent tissue residue depletion studies, were insufficiently described.

APPRAISAL

Depletion studies with thiazine ring radiolabeled ^{14}C-xylazine administered orally indicated that in rats 2% of radioactivity was still present 48 hours after administration. The ratio for recovery of the radiolabeled compound in urine and faeces was 7:3.

Pharmacokinetic data concerning the parent compound were reported in studies including cattle, horses, sheep, dogs and laboratory animals. Xylazine had a very short plasma half-life which in most species was

approximately 0.5 hours and in horses 0.9 hours. The compound underwent a rapid clearance. Species differences in clearance indicate different metabolic activity and/or different metabolic pathways. The apparent volume of distribution was large 1.9-2.5 l/kg due to the lipophilie nature of the drug. Plasma depletion of unlabeled compound in cattle was more rapid than depletion of total radioactivity in a similar study using ^{14}C-xylazine. Therefore, clarification of xylazine metabolism is required in order to better understand its pharmacokinetics.

The excretion of thiazine ring radiolabeled ^{14}C-xylazine administered intramuscularly to cattle (3 calves and one milking cow) and slaughtered at different time intervals was complete at 74 hours. The ratio of the radioactivity between urine and faeces was 3:1. In a related study using intramuscular administration of ^{14}C-xylazine labelled in the aniline ring, the excretion of the radioactivity was variable, ranging from 38-99%. In a second study, the respective ratios of the radioactivity for urine and faeces ranged from 12:1 to 6:1.

Studies on xylazine in rat and horse urine indicated extensive metabolism. However, no data concerning xylazine metabolism in other animals were available. Due to the lack of these data the possibility that metabolism causes the discrepancy between the depletion studies using radiolabeled compound and the unlabeled compound cannot be evaluated.

Two ^{14}C-radiolabel depletion studies using intramuscular administration of xylazine in cattle were submitted. The first study with three calves and one lactating cow used xylazine, labelled in the thiazine ring, and the second study used four calves and two lactating cows administered xylazine, labelled in the aniline ring. The radiolabeled tissue residue depletioo studies showed that total residues in mg/kg xylazine equivalents in kidney, liver, and injection site were 0.009-0.020, 0.022-0.050 and 0.030-1.152, respectively, at 72 hours after administration. Results were similar in the study using a thiazine ring labeled ^{14}C-xylazine. In milk the radioactivity as xylazine equivalents had declined to 0.01 mg/l after treatment with the drug labelled in the thiazine ring or in the aniline ring by 60 and 12 hours, respectively.

The data generated in tissues of cattle and milk residue depletion studies in which only the concentration of the parent compound, xylazine, was determined were in clear contrast with the radiolabel studies. The studies with unlabeled compound failed to detect xylazine at 0.01 mg/kg in muscle, kidney, liver and fat. Similarly, xylazine conceotrations in milk exceeded the 0.01 mg/l detection level only occasiooally. Thus the majority of the residues were not parent drug, but were unidentified metabolites.

A number of analytical methods, mainly for parent compound, such as photometry, liquid chromatography, gas chromatography, and mass spectrometry, were described. Performance chraracteristics were poorly determined but a limit of detection of 0.01 mg/kg was claimed. No method validation data were available for evaluation.

Maximum Residue Limits

The following factors were considered by the Committee with respect to the assignment of MRLs:

No ADI was established;
Lack of adequate data on metabolism of the compound;
No marker residue could be assigned; and
There were insufficient residue depletion studies available.

The Committee did not recommend MRLs.

The following informatioo would be required before a further review:

Data on xylazine metabolism in target species sufficient to identify a suitable marker residue and target tissues;

Additional data on residue depletion of xylazine and its metabolites in target species. These data should include evidence to show, in particular, whether 2,6-xylidine is present at the recommended withdrawal period; and

A suitable analytical method for determining the marker residue in target tissues.

REFERENCES

Etter, R., Battaglia, R., Noser, J. and Schuppischer, F., (1984). Nachweis von Tranquilizern in Nieren und Muskelfleiscb. Mitt. Gebiete Lebensm. Hyg., 75, 447-458.

Garcia-Villar, R., Toutain, P-L., Alvinerie, M. and Ruckebusch, Y., (1981). The pharmacokinetics of xylazine hydrochloride: An interspecific study. J. Vet. Pharmacol. Therap. 4, 87-92.

Dorn, H. and Maasfeld, M., (1990). Residue invesgations of tissue samples from cattle following treatment with Rompun. Bayer Study Report Dr.Mf./RA-77

Dorn, H. and Maasfeld, M., (1990h). Residue invesgations in milk samples from cows following treatment with Rompun. Bayer Study Report Dr.Mf./RA-78

Duhm, B., Maul, W., Medenwald, M., Patzschke, K. and Wagner, L., (1969). Untersuchungen mit radioactiv markiertem BAY Va 1470 an Ratten. Berliner und Muncbener Tierarztliche Wocbenschrift. 82, 104-109.

Heukamp, U., (1991a). Tissue concentrations of xylazine in calves following intramuscular administration of Rompun - analytical phase. Bayer Study Report BAY 160.

Heukamp, U., (1991b). Milk concentrations of xylazine in dairy cows following intramuscular administration of Rompun - analytical phase. Bayer Study Report BAY 161.

Keukens, H. and Aerts, M., (1989). Determination of carazolol and a number of tranquilizers in swine kidney by high performance liquid chromatography with ultraviolet and fluorescence detection. J. Chromatogr., 464, 149-161.

Maasfeld, W., (1991). Analytical method for the determination of xylazine in bovine tissues and milk. Bayer Study Report 76/1991.

Mutlih, A., Chui, Y., Young, L. and Abbott, F., (1992). Characterization of metabolites of xylazine produced in vivo and in vitro by LC/MS/MS and GC/MS. Drug Metab. Dispos. 20, 840-848.

Murphy, J. and Jacobs, K., (1975). Residues of Rompun and its metabolites in cattle. Bayer Study Report No. 43814, Bayer AG. Leverkusen, Germany.

Putter, J. and Sagner, G., (1969). BAY Va 1470. Residue examinations in muscles of sheep and cattle. Bayer Study Report 28018.

Putter, J. and Sagner, G., (1973). Chemical studies to detect residues of xylazine hydrochloride. Vet. Med. Rev. 2, 145-159.

Redgrave, V. and Cameron, D., (1991a). Tissue concentrations of xylazine in calves following intramuscular administration of Rompun - animal phase. Huntingdon Research Center Study Report BAY 160/91194.

Redgrave, V. and Cameron, D., (1991b). Milk concentrations of xylazine in dairy cows following intramuscular administration of Rompun - animal phase. Huntingdon Research Center Study Report BAY 160/91119.

Walker, D., (1995). Expert report on the residue file for xylazine (Rompun TS and 2%). Report ID 15570, Bayer AG. Leverkusen, Germany.

ANNEX I

SUMMARY OF JECFA EVALUATIONS OF VETERINARY DRUG RESIDUES FROM THE 32ND MEETING TO THE PRESENT

The attached table summarizes the veterinary drug evaluations conducted by JECFA at the 32nd (1987), the 34th (1989), the 36th (1990), the 38th (1991), the 40th (1992), the 42nd (1994), the 43rd (1994), 45th (1995) and 47th (1996) Meetings. These meetings were devoted exclusively to the evaluation of veterinary drug residues in foods. Please see Reports of those meetings, published in WHO Technical Report Series.

Some notes regarding the Table:

- The "Status" column refers to the ADI and indicates if "No" ADI was established, if a full ADI was given, or if the ADI is Temporary (TE).
- Where an MRL is temporary, it is so indicated by "TE".
- Several compounds have been evaluated more than once. The data given is for the most recent evaluation.

Substance	ADI (μg/kg bw)	ADI status	JECFA	MRL (μg/kg)	Tissue	Species	Marker residue (MR)
Abamectin	0-1[1]	Full	47 (1996)	100 50	Muscle, fat Kidney	Cattle	Avermectin B_{1a}
Albendazole	0-50	Full	34 (1989)	100[2] 5000[2]	Muscle, fat, milk Liver, kidney	Cattle, sheep	2-Aminobenzimidazole sulfone, MR in milk needs to be identified

[1]The ADI for abamectin was established by the 1995 Joint Meeting on Pesticide Residues (JMPR)

[2]Parent drug equivalents

Azaperone	0-3	TE	43 (1994)	60 TE 100 TE	Muscle, fat Liver, kidney	Pigs	Sum of azaperone and azaperol
Benzylpenicillin	30 μg/person/day	Full	36 (1990)	50 4	Muscle, liver, kidney Milk	All species	Parent drug
BST	Not specified	Full	40 (1992)	Not specified	Muscle, liver, kidney, fat, milk	Cattle	
Carbadox	Limited acceptance	Full	36 (1990)	30 5	Liver Muscle	Pigs	Quinoxaline-2-carboxylic acid
Carazolol	0-0.1	Full	43 (1994)	5[1] 25	Muscle, fat/skin Liver, kidney	Pigs	Parent drug
Ceftiofur	0-50	Full	45 (1995)	200 2000 4000 600 100 μg/l	Muscle Liver Kidney Fat Milk	Cattle, pigs Cattle	Desfuroylceftiofur
Chloramphenicol	None	No	42 (1994)	No MRL			
Chlorpromazine	None	No	38 (1991)	No MRL			
Chlortetracycline, oxytetracycline & tetracycline	0-3 (Group ADI)	Full	45 (1995) 47 (1996)	 100 300 600 200 100 100[2]	 Muscle Liver Kidney Eggs Milk Muscle	 Cattle, pigs, sheep, poultry Cattle, pigs, sheep, poultry Poultry Cattle, sheep Fish, Giant prawn	Parent drugs, singly or in combination

[1]The Committee noted that the concentration of carazolol at the injection site may exceed the ADI which is based on the acute pharmacological effects of carazolol

[2]Oxytetracycline only

Clenbuterol	0-0.004	Full	47 (1996)	0.2 0.6 0.05	Muscle, fat Liver, kidney Milk	Cattle, horses Cattle	Parent drug
Closantel	0-30	Full	36 (1990) 40 (1990)	 1000 3000 1500 5000 2000	 Muscle, liver Kidney, fat Muscle, liver Kidney Fat	 Cattle Sheep	Parent drug
Cypermethrin	0-50	Full	47 (1996)	200 TE 1000 TE 100 TE 50 TE	Muscle, liver, kidney Fat Eggs Milk	Cattle, sheep, chickens Chickens Cattle	Parent drug
α-Cypermethrin	0-20	Full	47 (1996)	100 TE 500 TE 50 TE 25 TE	Muscle, liver, kidney Fat Eggs Milk	Cattle, sheep, chickens Chickens Cattle	Parent drug
Dexamethasone	0-0.015	Full	42 (1992) 43 (1994)	0.5 TE 2.5 TE 0.3 μg/l TE	Muscle, kidney Liver Milk	Cattle, horses, pigs Cattle	Parent drug
Diclazuril	0-20	TE	45 (1995)	500 TE 3000 TE 2000 TE 1000 TE	Muscle Liver Kidney Fat	Sheep, rabbits & poultry	Parent drug
Dihydrostreptomycin & streptomycin	0-30 (Group ADI)	TE	43 (1994)	500 TE 1000 TE 200 μg/l TE	Muscle, liver, fat Kidney Milk	Cattle, pigs, chickens, sheep Cattle	Sum of dihydrostreptomycin and streptomycin
Dimetridazole	None	No	34 (1989)	No MRL			

Diminazene	0-100	Full	42 (1994)	500 12000 6000 150 μg/l	Muscle Liver Kidney Milk	Cattle	Parent drug
Doramectin	0-0.5	Full	45 (1995)	10[1] 100 30 150	Muscle Liver Kidney Fat	Cattle	Parent drug
Enrofloxacin	0-0.6	TE	43 (1994)	No MRL			
Estradiol-17β	Unnecessary	Full	32 (1987)	Unnecessary		Cattle	
Febantel	0-4[2]	TE	45 (1995)	100 TE 500 TE 100 μg/l TE	Muscle, kidney, fat Liver Milk	Cattle, sheep, pigs Cattle	Sum of fenbendazole, oxfendazole, and oxfendazole sulfone, expressed as oxfendazole sulfone equivalents
Fenbendazole (see febantel)							
Flubendazole	0-12	Full	40 (1992)	10 200 500 400	Muscle, liver Muscle Liver Eggs	Pigs Poultry	Parent drug
Flumequine	None	No	42 (1994)	No MRL			
Furazolidone	None	No	40 (1992)	No MRL			

[1]The Committee noted the high concentration of residues at the injection site during the 35-day period after parenteral administration of the recommended dose

[2]Group temporary ADI for febantel, fenbendazole and oxfendazole

Gentamicin	0-4	TE	43 (1994)	100 TE 200 TE 1000 TE 100 μg/l TE	Muscle, fat Liver Kidney Milk	Cattle, pigs Cattle	Parent drug
Ipronidazole	None	No	34 (1989)	No MRL			
Isometamidium	0-100	Full	40 (1992)	100 500 1000	Muscle, fat, milk Liver Kidney	Cattle	Parent drug
Ivermectin	0-1	Full	40 (1992) 36 (1990)	100 40 15 20	Liver Fat Liver Fat	Cattle Other species	H_2B_{1a}
Levamisole	0-6	Full	42 (1992)	10 100	Muscle, kidney, fat Liver	Cattle, sheep, pigs and poultry	Parent drug
Metronidazole	None	No	34 (1989)	No MRL			
Moxidectin	0-2	Full	45 (1995) 47 (1996)	 20[1] 50[1] 100 50 500	 Muscle Muscle Liver Kidney Fat	 Cattle, deer[2] Sheep Cattle, sheep, deer[2]	Parent drug

[1]At the 45th meeting the Committee noted the very high concentration and great variation in the level of residues at the injection site over a 49-day period after dosing cattle

[2]Temporary

Neomycin	0-60	Full	47 (1996)	500 10000 500 500 μg/l	Muscle, liver, fat Kidney Eggs Milk	Cattle, chickens, ducks, goats, pigs, sheep, turkeys Chickens Cattle	Parent drug
Nitrofurazone	None	No	40 (1992)	No MRL			
Olaquindox	Limited acceptance	TE	42 (1994)	No MRL but 4 μg/kg of MQCA (TE) is consistent with GVP	Muscle	Pigs	MQCA
Oxfendazole (see febantel)							
Oxolinic acid	None	No	43 (1994)	No MRL			
Oxytetracycline (see chlortetracycline)							
Progesterone	Unnecessary	Full	32 (1987)	Unnecessary		Cattle	
Propionylpromazine	None	No	38 (1991)	No MRL			
Ractopamine	None	No	40 (1992)	No MRL			
Ronidazole	Withdrawn	No	42 (1994)	No MRL			
Spectinomycin	0-40	Full	42 (1994)	300 TE 2000 TE 5000 TE 500 TE 200 μg/l TE	Muscle Liver Kidney Fat Milk	Cattle, pigs, and chicken	Parent drug

Spiramycin	0-50	Full	43 (1994) 47 (1996)	200* 200** 600* 600** 300* 300** 800* 300* 300** 100 µg/l*	Muscle Muscle Liver Liver Kidney Kidney Kidney Fat Fat Milk	Cattle, chickens Pigs Cattle, chickens Pigs Cattle Pigs Chickens Cattle, chickens Pigs Cattle	*Sum of spiramycin and neospiramycin **Expressed as spiramycin equivalents (antimicrobially active residues)
Streptomycin (see dihydrostreptomycin)							
Sulfadimidine	0-50	Full	42 (1994)	100 25 µg/l	Muscle, liver, kidney, fat Milk	Cattle, sheep, pigs and poultry Cattle	Parent drug
Sulphthiazole	None	No	34 (1989)	No MRL			
Testosterone	Unnecessary	Full	32 (1987)	Unnecessary		Cattle	
Tetracycline (see chlortetracycline)							
Thiamphenicol	0-6	TE	47 (1996)	40 TE	Muscle, liver, kidney, fat	Cattle, chickens	Parent drug
Tiabendazole	0-100	Full	40 (1992)	100 100	Muscle, liver, kidney, fat Milk	Cattle, pigs, goats and sheep Cattle, goats	Sum of tiabendazole and 5-hydroxytiabendazole
Tilmicosin	0-40	Full	47 (1996)	100 1000 1500 300 1000 50 TE	Muscle, fat Liver Liver Kidney Kidney Milk	Cattle, pigs, sheep Cattle, sheep Pigs Cattle, sheep Pigs Sheep	Parent drug

Trenbolone acetate	0-0.02	Full	34 (1989)	2 as MR 10 as MR	Muscle Liver	Cattle	β-Trenbolone α-Trenbolone
Triclabendazole	0-3	Full	40 (1992)	200 300 100 100	Muscle Liver, kidney Fat Muscle, liver, kidney, fat	Cattle Sheep	5-Chloro-6-(2',3'-dichlorophenoxy)-benzimidazole-2-one
Tylosin	None	No	38 (1991)	No MRL			
Xylazine	None	No	47 (1996)	No MRL			
Zeranol	0-0.5	Full	32 (1987)	2 10	Muscle Liver	Cattle	Parent drug

RECOMMENDATIONS ON COMPOUNDS EVALUATED BY THE 47TH JECFA

β-Adrenoceptor blocking agents

Clenbuterol

Acceptable daily intake (ADI): 0-0.004 μg per kg of body weight

Recommended maximum residue limits (MRLs)[1]

	Muscle (μg/kg)	Liver (μg/kg)	Kidney (μg/kg)	Fat (μg/kg)	Eggs (μg/kg)	Milk (μg/l)
Cattle	0.2	0.6	0.6	0.2		0.05
Horses	0.2	0.6	0.6	0.2		

[1]MRLs are expressed as the parent drug.

Xylazine

The Committee was unable to establish an ADI for xylazine because it concluded that a metabolite, 2,6-xylidine, is genotoxic and carcinogenic. The Committee was unable to establish MRLs for xylazine because of the lack of information on metabolism and residue depletion in edible tissues.

The following information would be required for further review:

- Data on xylazine metabolism in target species sufficient to identify a suitable marker residue and target tissues.
- Additional data on residue depletion of xylazine and its metabolites in target species. These data should include evidence to show, in particular, whether 2,6-xylidine is present at the recommended withdrawal times.
- A suitable analytical method for determining the marker residue in target tissues.

Anthelminthic agents

Abamectin

ADI: 0-1 μg per kg of body weight[1]

Recommended maximum residue limits (MRLs)[2]

	Muscle (μg/kg)	Liver (μg/kg)	Kidney (μg/kg)	Fat (μg/kg)	Eggs (μg/kg)	Milk (μg/l)
Cattle		100	50	100		

[1]This ADI, which applies to the parent drug abamectin, was established by the 1995 Joint FAO/WHO Meeting on Pesticide Residues (JMPR; FAO Plant Production and Protection Paper 133, 1996).
[2]MRLs are expressed as avermectin B_{1a}.

Moxidectin

ADI: 0-2 μg per kg of body weight[1]

Recommended maximum residue limits (MRLs)[2]

	Muscle (μg/kg)	Liver (μg/kg)	Kidney (μg/kg)	Fat (μg/kg)	Eggs (μg/kg)	Milk (μg/l)
Cattle	20	100	50	500		
Sheep	50[3]	100	50	500		
Deer[4]	20	100	50	500		

[1]This ADI was established at the forty-fifth meeting of the Committee.
[2]MRLs are expressed as the parent drug.
[3]This MRL was established at the present meeting. All other MRLs were established at the forty-fifth meeting of the Committee. At that meeting the Committee noted the high concentration and great variation in the level of residues at the injection site over a 49-day period after dosing cattle.
[4]Temporary MRLs (see the report of the forty-fifth meeting of the Committee).

Antimicrobial agents

Chlortetracycline, oxytetracycline and tetracycline

ADI: 0-3 μg per kg of body weight[1]

Recommended maximum residue limits (MRLs)[2]

	Muscle (μg/kg)	Liver (μg/kg)	Kidney (μg/kg)	Fat (μg/kg)	Eggs (μg/kg)	Milk (μg/l)
Cattle	100	300	600			100
Pigs	100	300	600			
Sheep	100	300	600			100
Poultry	100	300	600		200	
Giant prawn (*Penaeus monodon*)	100[3]					
Fish	100[3]					

[1]This ADI was established at the forty-fifth meeting of the Committee.
[2]MRLs are expressed as the parent drug, singly or in combination.
[3]This MRL applies only to oxytetracycline.

Neomycin

ADI: 0-60 μg per kg of body weight

Recommended maximum residue limits (MRLs)[1]

	Muscle (μg/kg)	Liver (μg/kg)	Kidney (μg/kg)	Fat (μg/kg)	Eggs (μg/kg)	Milk (μg/l)
Cattle	500	500	10 000	500		500
Pigs	500	500	10 000	500		
Sheep	500	500	10 000	500		
Goats	500	500	10 000	500		
Chickens	500	500	10 000	500	500	
Ducks	500	500	10 000	500		
Turkeys	500	500	10 000	500		

[1]MRLs are expressed as the parent drug.

Spiramycin

ADI: 0-50 μg per kg of body weight[1]

Recommended maximum residue limits (MRLs)

	Muscle (μg/kg)	Liver (μg/kg)	Kidney (μg/kg)	Fat (μg/kg)	Eggs (μg/kg)	Milk (μg/l)
Cattle[2]	200	600	300	300		100
Pigs[3]	200	600	300	300		
Chickens[2]	200	600	800	300		

[1]The ADI was established at the forty-third meeting of the Committee.
[2]MRLs are expressed as the sum of spiramycin and neospiramycin.
[3]MRLs are expressed as spiramycin equivalents (antimicrobially active residues).

Thiamphenicol

ADI: 0-6 μg per kg of body weight[1]

Recommended maximum residue limits (MRLs)[2]

	Muscle (μg/kg)	Liver (μg/kg)	Kidney (μg/kg)	Fat (μg/kg)	Eggs (μg/kg)	Milk (μg/l)
Cattle	40	40	40	40		
Chickens	40	40	40	40		

[1]Temporary ADI.
[2]Temporary MRLs, expressed as the parent drug.

The following information is required for evaluation in 1999:

- Detailed reports of the carcinogenicity study in rats on which the summary report was available at the present meeting and the range-finding study used to establish dose levels in that study.
- Residue depletion studies with radiolabelled and unlabelled thiamphenicol for identification of the marker residue and target tissues in non-ruminant cattle, chickens and pigs.

Tilmicosin

ADI: 0-40 μg per kg of body weight

Recommended maximum residue limits (MRLs)[1]

	Muscle (μg/kg)	Liver (μg/kg)	Kidney (μg/kg)	Fat (μg/kg)	Eggs (μg/kg)	Milk (μg/l)
Cattle	100	1000	300	100		
Pigs	100	1500	1000	100		
Sheep	100	1000	300	100		50[2]

[1]MRLs are expressed as the parent drug.
[2]Temporary MRL. The results of a study in lactating sheep with radiolabelled drug for estimation of the relationship between total residues and parent compound in milk are required for evaluation in 1999.

Insecticides

Cypermethrin

ADI: 0-50 μg per kg of body weight

Recommended maximum residue limits (MRLs)[1]

	Muscle (μg/kg)	Liver (μg/kg)	Kidney (μg/kg)	Fat (μg/kg)	Eggs (μg/kg)	Milk (μg/l)
Cattle	200	200	200	1000		50
Sheep	200	200	200	1000		
Chickens	200	200	200	1000	100	

[1]Temporary MRLs, expressed as the parent drug.

The following information is required for evaluation in 2000:

- The results of radiodepletion studies that extend beyond the recommended withdrawal times using the drug in its topical formulation. The study should determine the depletion of the total residues and the parent drug in target species.
- Evidence to verify that no interconversion of isomeric forms occurs during metabolism in the target species.
- Further information on the validation of analytical methods, particularly data on the derivation of the limits of determination and limits of quantification.

***alpha*-Cypermethrin**

ADI: 0-20 μg per kg of body weight

Recommended maximum residue limits (MRLs)[1]

	Muscle (μg/kg)	Liver (μg/kg)	Kidney (μg/kg)	Fat (μg/kg)	Eggs (μg/kg)	Milk (μg/l)
Cattle	100	100	100	500		25
Sheep	100	100	100	500		
Chickens	100	100	100	500	50	

[1]Temporary MRLs, expressed as the parent drug.

The following information is required for evaluation in 2000:

- The results of radiodepletion studies in sheep and chickens that extend beyond the recommended withdrawal times using the drug in its topical formulation. The study should determine the depletion of the total residues and the parent drug.
- The radiodepletion study submitted for cattle should be reassessed to determine the depletion of the total residues and the parent drug.
- Evidence to verify that no interconversion of the *cis*-isomeric forms to the *trans*-isomeric forms occurs during metabolism in the target species.
- Further information on the validation of analytical methods, particularly data on the derivation of the limits of determination and limits of quantification.

RESIDUES OF SOME VETERINARY DRUGS IN ANIMALS AND FOODS
FAO FOOD AND NUTRITION PAPERS
41/4, 41/5, 41/6, 41/7, AND 41/8
ROME 1991, 1993, 1994, 1995, 1996, RESPECTIVELY

CORRIGENDUM

The Tables in the above FNPs - Summary of JECFA Evaluations of Veterinary Drug Residues from the 32nd Meeting to the Present are replaced by a similar table on page 127 of this publication

FAO TECHNICAL PAPERS

FAO FOOD AND NUTRITION PAPERS

1/1	Review of food consumption surveys 1977 – Vol 1. Europe, North America, Oceania, 1977 (E)
1/2	Review of food consumption surveys 1977 – Vol 2. Africa, Latin America, Near East, Far East, 1979 (E)
2	Report of the joint FAO/WHO/UNEP conference on mycotoxins, 1977 (E F S)
3	Report of a joint FAO/WHO expert consultation on dietary fats and oils in human nutrition, 1977 (E F S)
4	JECFA specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers, 1978 (E)
5	JECFA – guide to specifications, 1978 (E F)
5 Rev 1	JECFA – guide to specifications, 1983 (E F)
5 Rev. 2	JECFA – guide to specifications, 1991 (E)
8	The feeding of workers in developing countries, 1976 (E S)
7	JECFA specifications for identity and purity of food colours, enzyme preparations and other food additives, 1978 (E F)
8	Women in food production, food handling and nutrition, 1979 (E F S)
9	Arsenic and tin in foods: reviews of commonly used methods of analysis, 1979 (E)
10	Prevention of mycotoxins, 1979 (E F S)
11	The economic value of breast-feeding, 1979 (E F)
12	JECFA specifications for identity and purity of food colours, flavouring agents and other food additives, 1979 (E F)
13	Perspective on mycotoxins, 1979 (E F S)
14	*Manuals of food quality control.*
14/1	Food control laboratory, 1979 (Ar E)
14/1 Rev 1	The food control laboratory, 1986 (E)
14/2	Additives, contaminants, techniques, 1980 (E)
14/3	Commodities, 1979 (E)
14/4	Microbiological analysis, 1979 (E F S)
14/5	Food inspection, 1981 (Ar E) (Rev. 1984, E S)
14/6	Food for export, 1979 (E S)
14/6 Rev.1	Food for export, 1990 (E S)
14/7	Food analysis: general techniques, additives, contaminants and composition, 1986 (C E)
14/8	Food analysis: quality, adulteration and tests of identity, 1986 (E)
14/9	Introduction to food sampling, 1988 (Ar C E F S)
14/10	Training in mycotoxins analysis, 1990 (E S)
14/11	Management of food control programmes, 1991 (E)
14/12	Quality assurance in the food control microbiological laboratory, 1992 (E F S)
14/13	Pesticide residue analysis in the food control laboratory, 1993 (E F)
14/14	Quality assurance in the food control chemical laboratory, 1993 (E)
14/15	Imported food inspection, 1993 (E F)
14/16	Radionuclides in food, 1994 (E)
15	Carbohydrates in human nutrition, 1980 (E F S)
16	Analysis of food consumption survey data for developing countries, 1980 (E F S)
17	JECFA specifications for identity and purity of sweetening agents, emulsifying agents, flavouring agents and other food additives, 1980 (E F)
18	Bibliography of food consumption surveys, 1981 (E)
18 Rev 1	Bibliography of food consumption surveys, 1984 (E)
18 Rev. 2	Bibliography of food consumption surveys, 1987 (E)
18 Rev 3	Bibliography of food consumption surveys, 1990 (E)
19	JECFA specifications for identity and purity of carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents and other food additives, 1981 (E F)
20	Legumes in human nutrition, 1982 (E F S)
21	Mycotoxin surveillance – a guideline, 1982 (E)
22	Guidelines for agricultural training curricula in Africa, 1982 (E F)
23	Management of group feeding programmes, 1982 (E F P S)
23 Rev 1	Food and nutrition in the management of group feeding programmes, 1993 (E F S)
24	Evaluation of nutrition interventions, 1982 (E)
25	JECFA specifications for identity and purity of buffering agents, salts, emulsifiers, thickening agents, stabilizers; flavouring agents, food colours, sweetening agents and miscellaneous food additives, 1982 (E F)
26	Food composition tables for the Near East, 1983 (E)
27	Review of food consumption surveys 1981, 1983 (E)
28	JECFA specifications for identity and purity of buffering agents, salts, emulsifiers, stabilizers, thickening agents, extraction solvents, flavouring agents, sweetening agents and miscellaneous food additives, 1983 (E F)
29	Post-harvest losses in quality of food grains, 1983 (E F)
30	FAO/WHO food additives data system, 1984 (E)
30 Rev. 1	FAO/WHO food additives data system, 1985 (E)
31/1	JECFA specifications for identity and purity of food colours, 1984 (E F)
31/2	JECFA specifications for identity and purity of food additives, 1984 (E F)
32	Residues of veterinary drugs in foods, 1985 (E/F/S)
33	Nutritional implications of food aid an annotated bibliography, 1985 (E)
34	JECFA specifications for identity and purity of certain food additives, 1986 (E F)
35	Review of food consumption surveys 1985, 1986 (E)
36	Guidelines for can manufacturers and food canners, 1986 (E)
37	JECFA specifications for identity and purity of certain food additives, 1986 (E F)
38	JECFA specifications for identity and purity of certain food additives, 1988 (E)
39	Quality control in fruit and vegetable processing, 1988 (E F S)
40	Directory of food and nutrition institutions in the Near East, 1987 (E)
41	Residues of some veterinary drugs in animals and foods, 1988 (E)
41/2	Residues of some veterinary drugs in animals and foods. Thirty-fourth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1990 (E)

41/3 Residues of some veterinary drugs in animals and foods. Thirty-sixth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1991 (E)

41/4 Residues of some veterinary drugs in animals and foods. Thirty-eighth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1991 (E)

41/5 Residues of some veterinary drugs in animals and foods. Fortieth meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1993 (E)

41/6 Residues of some veterinary drugs in animals and foods. Forty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1994 (E)

41/7 Residues of some veterinary drugs in animals and foods. Forty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1994 (E)

41/8 Residues of some veterinary drugs in animals and foods. Forty-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1998 (E)

41/9 Residues of some veterinary drugs in animals and foods. Forty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1997 (E)

42 Traditional food plants, 1988 (E)

42/1 Edible plants of Uganda. The value of wild and cultivated plants as food, 1989 (E)

43 Guidelines for agricultural training curricula in Arab countries, 1988 (Ar)

44 Review of food consumption surveys 1988, 1988 (E)

45 Exposure of infants and children to lead, 1989 (E)

46 Street foods, 1990 (E/F/S)

47/1 Utilization of tropical foods: cereals, 1989 (E F S)

47/2 Utilization of tropical foods: roots and tubers, 1989 (E F S)

47/3 Utilization of tropical foods: trees, 1989 (E F S)

47/4 Utilization of tropical foods. tropical beans, 1989 (E F S)

47/5 Utilization of tropical foods: tropical oil seeds, 1989 (E F S)

47/6 Utilization of tropical foods: sugars, spices and stimulants, 1989 (E F S)

47/7 Utilization of tropical foods: fruits and leaves, 1990 (E F S)

47/8 Utilization of tropical foods: animal products, 1990 (E F S)

48 Number not assigned

49 JECFA specifications for identity and purity of certain food additives, 1990 (E)

50 Traditional foods in the Near East, 1991 (E)

51 Protein quality evaluation. Report of the Joint FAO/WHO Expert Consultation, 1991 (E,F)

52/1 Compendium of food additive specifications – Vol. 1, 1993 (E)

52/2 Compendium of food additive specifications – Vol. 2, 1993 (E)

52 Add. 1 Compendium of food additive specifications – Addendum 1, 1992 (E)

52 Add. 2 Compendium of food additive specifications – Addendum 2, 1993 (E)

52 Add 3 Compendium of food additive specifications – Addendum 3, 1995 (E)

52 Add. 4 Compendium of food additive specifications, 1996 (E)

53 Meat and meat products in human nutrition in developing countries, 1992 (E)

54 Number not assigned

55 Sampling plans for aflatoxin analysis in peanuts and corn, 1993 (E)

56 Body mass index – A measure of chronic energy deficiency in adults, 1994 (E F S)

57 Fats and oils in human nutrition, 1995 (E F S)

58 The use of hazard analysis critical control point (HACCP) principles in food control, 1995 (E F S)

59 Nutrition education for the public, 1995 (E F S)

60 Food fortification: technology and quality control, 1996 (E)

61 Biotechnology and food safety, 1996 (E)

62 Nutrition education for the public – Discussion papers of the FAO Expert Consultation, 1996 (E)

63 Street foods, 1997 (E/F/S)

64 Worldwide regulations for mycotoxins 1995 – A compendium, 1997 (E)

Availability: march 1997

Ar – Arabic
C – Chinese
E – English
F – French
P – Portuguese
S – Spanish

Multil – Multilingual
* Out of print
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This document is one of three publications prepared by the forty-seventh session of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Rome in June 1996 and dedicated exclusively to the evaluation of veterinary drug residues in foods. The report of the meeting will be published in the WHO Technical Report Series, and the toxicological monographs as No. 38 in the WHO Food Additives Series. Residue monogrsphs in this document provide information on chemicsl identity, properties, use, pharmscokinetics, metabolism, tissue residus depletion snd snslytical methods for subetances indicsted on the cover. The publication is meant for regulstory authorities, veterinsry drug researchsrs snd sny other concerned persons who wish to gain informstion snd insight into the needs snd problems involved in establishing maximum limits for veterinary drug residues in food.

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